GENETICS

A PERIODICAL RECORD OF INVESTIGATIONS BEARING ON HEREDITY AND VARIATION

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by

GEORGE H. SHULL

with the cooperation of

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ERICH VON TSCHERMAK-SEYSENEGG

IN September 1950 this journal cooperated with the Genetics Society of America, the American Society of Naturalists, and the American Society of Human Genetics in a four-day celebration of the "Golden Jubilee of Genetics—the first fifty years." The special contribution of GENETICS to this celebration was the publication of a supplement containing translations into English of ten highly significant letters of GREGOR MENDEL, written to CARL NÄGELI, and the three papers of DE VRIES, CORRENS and TSCHERMAK through which the work of MENDEL was confirmed and brought to general notice. The triple announcement of this essentially simultaneous "rediscovery" and confirmation of the Mendelian principles is legitimately accepted as marking the birth-date of the science of genetics, which received its christening in 1906 at the suggestion of WILLIAM BATESON, President of the Third International Congress on Hybridization, held in London. Now, little more than a year after the "Golden Jubilee" celebration, it becomes our privilege to extend to the last surviving "rediscoverer" warm felicitations on the attainment of his eightieth birthday, and to present herewith, while the revered subject is still with us, although no longer in perfect health and characteristic vigor, a recent photograph as the frontispiece of Volume 37.

The writer of this sketch treasures the memories of life-long mutual personal friendships with all three of these great "rediscoverers," and recalls with satisfaction his first meeting of ERICH VON TSCHERMAK on the evening of November 6, 1908, when, after a memorable day at the Königskloster of ALT BRÜNN as dinner guest of FATHER BAŘINA, MENDEL's successor as Prelate of the Monastery, he arrived by train after dusk at the station in Vienna, wondering how he would ever find DOCTOR VON TSCHERMAK in the milling crowds, but the latter did the finding on the basis of a photograph he had seen. During the next five memorable days the writer occupied ERICH's own commodious and well furnished room and slept in ERICH's big comfortable bed while his host betook himself to a pallet in the narrow confines of a small cubicle that normally served as a house-maid's quarters. From the VON TSCHERMAK home as a center, excursions were made to all of the institutions of biological and breeding interest accessible in and from Vienna, most important of all being ERICH's own place of work, the Hochschule für Bodenkultur, where the visitor's attention was called pridefully to the inscription on the door of his lecture-room, "Lehrkanzel für Pflanzenzüchtung," the first lecture hall in Europe, perhaps the first in the world, to bear such a title. One evening there was a special dinner in the VON TSCHERMAK home with all members of the family assembled, thus giving opportunity to meet ERICH's sister SILVIA VON TSCHERMAK, already known for her work in the field of colloid chemistry, and his elder brother ARMIN VON TSCHERMAK, Professor of Physiology in the German University of Prag, Czechoslovakia, whom

ERICH has always considered his best teacher and constant collaborator. The parents, HOFRAT PROFESSOR DOCTOR GUSTAV VON TSCHERMAK, the renowned mineralogist, and his wife HERMINE VON TSCHERMAK, née FENZL, were most gracious host and hostess, and the occasion has left an indelible impression.

On this visit occasion was also taken to consider together the details of the contemplated first visit to America of ERICH and his distinguished teacher and friend, PROFESSOR DOCTOR KURT VON RÜMKER, of Breslau, Germany, which ensued the following April to August, 1909, and took the travelers to most of the leading experiment stations and other institutions at which genetical and practical breeding operations were in progress, from Boston, New Haven, Cold Spring Harbor, and Washington, D. C., to San Francisco, Berkeley and Santa Rosa, California, and Seattle, Washington, and from Ottawa, Canada, St. Paul, Minnesota, and Fargo, North Dakota, to Tucson, Arizona. The fully documented and competently analyzed report of this survey of genetical and breeding activities in North America was published in Berlin in 1910 and served to acquaint European breeders with what was going on in this field on this side of the Atlantic. It had a wide distribution and was promptly translated into Russian.

My next meeting of ERICH VON TSCHERMAK was at the Carmel Farm of the Connecticut Agricultural Experiment Station, where I went on July 21, 1909, for my first direct observations of the corn breeding experiments of DOCTOR EDWARD MURRAY EAST, and to connect with the two distinguished travelers and accompany them by steamer across Long Island Sound and to Cold Spring Harbor, for their visit to the Station for Experimental Evolution, thus avoiding the complicated transfer by way of New York City. In 1910 DOCTOR VON TSCHERMAK made his second and last visit to America, when he lectured at Ames, Iowa, on breeding cereal grains, but this time he was in America only fourteen days and visited no other breeding or research institutions.

HOFRAT PROFESSOR DR. H. C. DR. PHIL. ERICH VON TSCHERMAK-SEYSENEGG was born in Vienna on November 15, 1871, the youngest of four children in an intellectually gifted family. His father was the distinguished mineralogist, PROFESSOR GUSTAV VON TSCHERMAK (b. 1836, d. 1927), and his maternal grandfather was the famous botanist, EDUARD FENZL (b. 1808, d. 1879), Professor of Botany in the University and Director of the Botanical Garden in Vienna. The appellation SEYSENEGG is a restoration of a similar appellation borne by the ancestors of PROFESSOR FENZL and was adopted by ERICH'S father when he was ennobled at the time of his pensioning in 1907. After completing the course of study in the Gymnasium in Kremsmünster, Ober Oesterreich, ERICH spent one year taking courses simultaneously in the University of Vienna and the Hochschule für Bodenkultur, and then interrupted his formal schooling on the advice of his Botany Professor, J. BOEHM, in order to gain practical experience in agricultural procedures on the Rotvorwerk Farm near Freiberg, Saxony. He then entered the University of Halle a. S. where he completed his formal agricultural studies as a student

of the great plant physiologists, JULIUS KÜHN and MAX MAERKER, and won his doctorate in the spring of 1896 with a thesis under the botanist GREGOR KRAUS. In 1901 his doctorate was validated by the University of Vienna.

Entertaining at first the intention to make a career in the practical breeding of vegetables, flowers and orchard fruits, he spent two years as a volunteer worker at the seed-breeding establishments of CHRISTIAN BERTRAM in Stendal, and of DAVID SACHS, DIPPE, and METTE, at Quedlinburg, and also visited the renowned grain-breeding establishments in Saxony, especially that of the celebrated AMSTRAT DR. H. C. WILHELM RIMPAU at Schlanstedt. Returning to Vienna he gave a lecture on January 7, 1898, before the Club der Land- und Forstwirte, which so impressed PROFESSOR A. VON LIEBENBERG of the Hochschule für Bodenkultur that he offered TSCHERMAK an Assistantship in that institution as soon as a vacancy should occur, and although he was obliged to wait several years for the fulfillment of this promise, he finally became an Assistant in PROFESSOR VON LIEBENBERG's department in 1901, with minimal requirement of service in order that he might have time for his own investigations. Although handicapped by inadequate financial support and being obliged often to perform the work of an ordinary laborer, which should have been provided by a more sympathetic Administration, he remained all the rest of his life a member of the faculty of the Hochschule für Bodenkultur, notwithstanding many attractive offers from other institutions which sought to secure his services. His strong attachment to his family, his neighbors, his neighborhood, his city and his native land was a strongly determining factor in his character, and he preferred to struggle along with the limited facilities provided in Vienna to the acceptance of the opportunities and responsibilities involved in larger and better known institutions to which he was invited in foreign countries. Under pressure of these many offers from other institutions his advancement in grade in the Hochschule für Bodenkultur was exceptionally rapid, and he was promoted to a. o. Professor in 1906, and to Ordinarius in 1909, attained the dignity and the title of Hofrat in 1921, and finally Doctor *honoris causa* from his own institution in 1948.

While waiting somewhat impatiently for the promised connection with the Hochschule in Vienna, DR. TSCHERMAK acted on the advice of the distinguished Belgian mineralogist and Jesuit priest, FATHER RENARD, a former student of his father, that he come to Ghent and expand his horticultural knowledge and experience by learning the techniques of the Belgian flower growers. He went to Ghent in the spring of 1898, but was disappointed to find that he could only gain familiarity with greenhouse management, not with breeding techniques with vegetables and garden flowers as he had hoped. Having time on his hands, he arranged to carry on breeding experiments in the Botanical Garden of Ghent and worked out a program with the DIRECTOR DR. J. MACLEOD for experiments on different types of breeding in relation to heterosis and xenia effects as manifested in the garden pea when different varieties are crossed having different colors and forms of seeds. His choice of *Pisum sativum* for these experiments was determined largely by two con-

siderations: he had plans to spend that summer, from July on, in visiting breeding stations in England, Holland and France, and consequently needed a plant of short period of growth; also he was impressed by the inadequacy of data cited in DARWIN's "Effects of cross and self fertilisation in the vegetable kingdom" (1877) which based conclusions with respect to peas on a comparison of only four pairs of plants, four plants from selfed parents and four from crosses.

The next year, at PROFESSOR VON LIEBENBERG's suggestion he became a voluntary worker at the Imperial Family's Foundation at Esslingen, near Vienna, in order to prepare himself for the management of an experiment station which it was proposed to establish in the near future at Gross Enzersdorf for the use of the Hochschule für Bodenkultur. While thus engaged he continued his cultures of peas in the private garden of a local banker, and on recording the data from these cultures was impressed by the recurrence of 3:1 ratios of yellow and green seeded peas and of smooth and wrinkled seeded peas when the crossed parents were self fertilized, and the 1:1 ratios when green seeded and wrinkled seeded plants were fertilized with pollen from the cross-bred plants. In preparing to use these results as the basis for his professional qualifying thesis "Ueber künstliche Kreuzung bei *Pisum sativum*," he ran across the rather ambiguous reference to MENDEL's work with peas in FOCKE's "Pflanzenmischlinge" (1881), and had MENDEL's paper sent to him from the University of Vienna library, and thus learned to his great surprise that MENDEL had already performed the same experiments with much greater comprehensiveness than his own, had discovered the same regular relationships, and had adequately elaborated their statistical significance. TSCHERMAK finished his paper by Christmas, 1899, and it was accepted and was read as his inaugural dissertation on January 17, 1900, before the Rectory of the Hochschule für Bodenkultur. Not until the appearance of DE VRIES's announcement "Sur la loi de disjonction des hybrides" in the French Compte Rendus for March, 1900, followed almost immediately by a similar German announcement by the same author in the Berichte der deutschen botanischen Gesellschaft, did TSCHERMAK learn that MENDEL's discoveries were also known to another contemporary worker. Naturally he was very much disturbed, and even more so when there followed immediately afterward the rejoinder from DOCTOR CORRENS announcing that he too had simultaneously reached the same conclusions from his work with peas and maize. He had very good reason to be disturbed, as he was very considerably younger than the other two "rediscoverers,"—seven years younger than CORRENS and 23 years younger than DE VRIES. Both DE VRIES and CORRENS had already won distinction by published works of profound and universally recognized significance, while for TSCHERMAK this was his first bid for scientific recognition. Furthermore, his dissertation was much too long, some 90 pages, for publication in the Berichte der deutschen botanischen Gesellschaft, where the brief reports of the other two had found very prompt publication. In view of his inexperience in matters of publication this was a real dilemma, but

his friend DOCTOR BERSCH came to the rescue and the paper was accepted for immediate publication in the Zeitschrift für das landwirtschaftlichen Versuchswesen in Oesterreich, and by special arrangement the reprints were prepared in advance of the publication of the journal. He also prepared a seven page abstract for the Berichte d. d. bot. Gesell. in which it was published in June; but the preprinted copies of the complete work were distributed in May, 1900, a month ahead of the abstract. In this way the handicaps to full and prompt recognition were somewhat successfully overcome and the three rediscoverers came to be in time placed on equal pedestals of renown in the history of biological progress.

Of this trio of "rediscoverers" TSCHERMAK was quite understandably the only one who was impressed with the significance of the Mendelian discoveries for practical breeding procedures, since his entire background of preparation had been oriented toward practical agricultural and horticultural plant breeding. On a visit to Svalöf, Sweden, in 1901, he urged the revolutionary importance of these new discoveries so successfully that he has always been credited as the initiator of the practical utilization of the Mendelian concepts and methods at that great seed-breeding laboratory. Even before the end of 1900 he urged the republication of MENDEL's basic paper in OSTWALD's "Klassiker der exakten Wissenschaften" and although this effort met with opposition at first it achieved success the following year. Another contribution of great significance was the publication in 1903 of an important paper on "Die Theorie der Kryptomerie und des Kryptohybridismus" which served to clarify the often quite complicated inheritance ratios and apparent exceptions from Mendelian "expectations," as exemplified by the frequent occurrence of so-called "novelties," and to bring them into line with the typical simple Mendelian cases.

His most notable contributions have been made in the breeding of the cereal grains with which he began work in 1899 at Esslingen and published his first paper on this subject in 1901 in the same journal that had published his dissertation the preceding year. By 1906 he had become a widely recognized authority to whom PROF. DR. C. FRUWIRTH gladly assigned the preparation of the sections on correlation and hybridization in the several cereal grains and in sugar beets in the fourth volume of his great encyclopedic work, "Handbuch der landwirtschaftlichen Pflanzenzüchtung." In the second edition of this fundamental work, published in 1910, DOCTOR VON TSCHERMAK prepared more than three-eighths of the contents, and in the fourth edition, published in 1923, and bearing the title "Die Züchtung der vier Hauptgetreidearten und der Zuckerrübe," prepared in collaboration with DR. FRUWIRTH and DR. TH. ROEMER, DR. VON TSCHERMAK contributed almost 45 per cent of the contents of the volume, taking care of all sections dealing with correlations and hybridization in each of the major crop grains, wheat, rye, barley and oats. In 1913 he published the first of many reports on his remarkable constant and fertile species hybrids and intergeneric hybrids in the cereals, which he designated "additive hybrids" in contra-distinction to the typical

splitting hybrids of MENDEL's peas. MENDEL's Hieracium hybrids were of the "additive" type. Of greatest significance were the wheat × rye (Triticale), rye × wheat (Secalotricum), wheat–aegilops hybrids (Aegilotricum), wheat–*Haynaldia villosa* hybrids (Haynaldtricum), wheat–couch grass hybrids (Agrotricum) and rye–couch grass hybrids (Agrocale). The cytological situation in these hybrids was worked out by two of his students, BLEIER and VON BERG, and through these studies his theory of chromosome addition without interchange or splitting as the explanation of the intermediacy, constancy and full fertility of such hybrids was fully authenticated, and has served as a basis for understanding the experiences of many previous and contemporary observers.

Closely related to these studies of species hybrids and intergeneric hybrids has been VON TSCHERMAK's demonstration of artificially induced parthenocarpy and parthenogenesis, first observed by him in certain wide crosses in several genera of Leguminosae which he had under observation at Ghent in 1898 and resumed and extended during the last fifteen years. Since 1936 he has performed many experiments by the application of completely unrelated pollen, as well as vitamin-containing powders, and finally, merely water-extracting powders, to the stigmas of different species of plants with positive results that have been described in a number of recent reports.

Besides prosecuting his own experimental work with characteristic vigor, he has been very active in promoting experimental and practical breeding in Austria and Czechoslovakia by founding many breeding stations, especially throughout Lower Austria and also in Bohemia and Moravia. With the cooperation of DOCTOR FRUWIRTH he founded in 1912 the Gesellschaft für Pflanzenzüchtung in Wien (known as "Z") which has provided for its members many stimulating lectures and interesting excursions to places of breeding interest both in Austria and in foreign lands. In his own application of the Mendelian principles to practical breeding, to which he devoted much attention after the close of the first World War, he produced many varieties of high-yielding gluten-rich winter and summer wheats, valuable winter and summer barleys, oats, peas and stringless beans, many of which bear his name, and which have materially increased the yields and improved the qualities of these field crops and garden vegetables, and have won for him many medals and other forms of public recognition. He has been particularly interested in the potentialities of a bush-gourd he has produced with thin-shelled oily seeds which offer a valuable source of vegetable oil and may find a market as a substitute for almonds. Among his floral productions a strain of colorful calycanthemous and double-flowered primulas has given him much pleasure, but these were unfortunately lost as a war casualty.

Since the death of HUGO DE VRIES on May 21, 1935, he has been for more than sixteen years the sole surviving member of the trio of rediscoverers, CARL CORRENS having passed away on February 14, 1933. Having soared almost at a single leap to a position of world renown at the early age of 29 years, TSCHERMAK accepted the challenge with commendable modesty and

by notable and unremitting industry has continued to carve for himself a unique and permanent place in the annals of genetics, for which he has received so many honors that a mere catalog of them would be too long for inclusion here. I mention only that in 1924 he received from the Landwirtschaftlichen Hochschule in Berlin the first of his five honorary doctorates, the last two of which, as is commonly the case, having been given him in belated final local recognition, by his own institution, the Hochschule für Bodenkultur, in 1948, and by the University of Vienna in 1950. He was elected a Corresponding Member of the Austrian Academy of Sciences in 1926 and advanced to full Member in 1930. He is likewise Member, Corresponding Member or Honorary Member of eight foreign National Academies of Science, and Corresponding Member or Honorary Member of many other foreign scientific and learned societies. Among his many medals are the Cothenius medal of the Akademie der Naturforscher in Halle a. S. and the Goethe medal.

ERICH VON TSCHERMAK seemed wedded to his research program and remained a bachelor until rather late in life, but found a kindred spirit in FRÄULEIN LOTTKA VON NIEBAUER (1876–1950) whom he married on August 4, 1926, when both were in their fifties. This proved a rewarding and happy union, and they strove to emulate the consistent tradition of ERICH's parents and his sister SILVIA by making their home a place of culture and refinement where their many friends found always a warm welcome and opportunity for intellectual and uplifting social intercourse; but after more than two decades of happy companionship, tragedy came in the year-long illness of LOTTKA VON TSCHERMAK, characterized by unremitting, heart-breaking suffering which ended mercifully on July 5, 1950.

The Editorial Board of GENETICS, speaking also confidently for all readers of GENETICS, offers hereby our cordial felicitations to ERICH VON TSCHERMAK-SEYSENEGG on the attainment of his eightieth birthday and the continuation of a life replete with great and good deeds for the promotion of genetical science and the welfare of humankind. We recognize in him a shining example and an inspiring symbol and wish for him a long enjoyment of the youthful spirit that finds ringing expression in the closing sentence of his recent survey * of the first fifty years of Mendelism: "Wir stellen unsere Arbeit nicht ein, im Gegenteil wir bauen weiter"!

GEORGE H. SHULL

* TSCHERMAK-SEYSENEGG, E. v., 1951 Historischer Rückblick auf die Wiederentdeckung der Gregor Mendelschen Arbeit. Verhandl. d. Zool.-Bot. Gesell. in Wien 92 (100-Jahr-Festschr.): 25–35.

SPONTANEOUS CHROMOSOME BREAKAGE AND ATYPICAL CHROMOSOME MOVEMENT IN MEIOSIS OF THE HYBRID BROMUS MARGINATUS × B. PSEUDOLAEVIPES

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University of California, Santa Barbara, California

Received February 26, 1951

INTRODUCTION

SPONTANEOUS chromosome breakage, apparently identical with that produced by irradiation, has recently been reported in meiosis of an interspecific hybrid, *Bromus Trinii* × *B. maritimus* (WALTERS 1950). The present hybrid, *Bromus marginatus* × *B. pseudolaevipes,* also exhibits fragments at meiosis which indicate the occurrence of chromosome breakage in this division. However, the fragments observed in this hybrid indicate a type of breakage different from that reported previously. An analysis of this type of chromosome breakage was one objective of the present investigation.

Metaphase bivalents with thin regions between centromeres and the chiasma are found in the present hybrid and in some other hybrids of Bromus, in whose parent species the bivalent chromosomes are relatively constant in diameter. A second objective of this investigation was the description of the bivalents exhibiting various thin regions, and a discussion of their origin.

Chromosome movement in meiotic anaphase by means of terminal secondary centromeric activity has been described in Zea (RHOADES and VILKOMERSON 1942) and Secale (ÖSTERGREN and PRAKKEN 1946). The present hybrid, in which certain chromatid ends appear to be drawn towards the poles at anaphase, may represent a third example of chromosome movement by means of centromeric activity of terminal regions. A description and discussion of the chromosomes exhibiting this aberrant movement was the final objective of the present study.

MATERIALS AND METHODS

The relationships of the species, whose hybrid is described here, are discussed by STEBBINS (1947). *Bromus marginatus* (2n = 56) is placed in the section Ceratochloa, *B. pseudolaevipes* (2n = 14) is in the section Bromopsis. The hybrid *B. marginatus* × *B. pseudolaevipes* was grown in the field in Berkeley in 1946, and fixations from two plants were made at that time. The original collection of *B. marginatus* was made at Meadow View Ranger Station, Plumas County, California, and that of *B. pseudolaevipes* from north of Castaic, Los Angeles County; fixations for meiotic studies were made from plants grown in the field in Berkeley in 1950.

Fixations for cytological study were made in a mixture of 3 parts absolute alcohol : 1 part glacial acetic acid and stored in 70 percent alcohol. Meiosis

was studied in iron-acetocarmine smears of microsporocytes. One anther of the hybrid material was sectioned at 8 μ and stained with STOCKWELL's (1934) triple stain.

MEIOSIS IN THE PARENTS

Meiosis was studied in three different plants of *B. pseudolaevipes* and two of *B. marginatus*.

B. marginatus has seven pairs of large chromosomes and 21 pairs of small chromosomes; within these groups the chromosomes are not distinguishable in meiosis (STEBBINS and TOBGY 1944). Metaphase bivalents generally have two or three chiasmata, and the diameter of these chromosomes is relatively

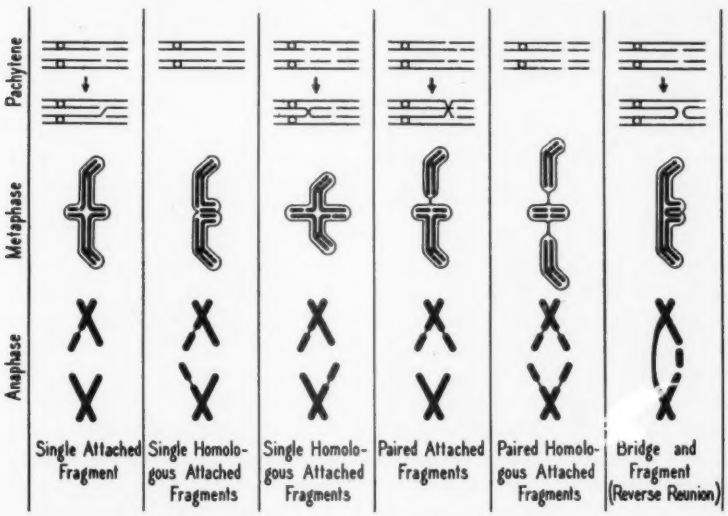

FIGURE 1.—Diagrams showing different types of attached fragments observed in the hybrid and their postulated origin by cross-over breaks.

constant (fig. 2). Certain irregularities were observed in first anaphase: In many cells the chromosomes are very irregular in outline and have an "eroded" appearance; in extreme cases portions of chromosome arms are thread-like, sometimes appearing similar to attached fragments. In about 24 percent of the early- and mid-anaphase cells examined, one or more bivalents showed delayed separation or difficulty of separation, apparently because of slight stickiness. Constrictions are found and sometimes an attached fragment is observed (fig. 3). Rarely a similar attached fragment is observed on each of two chromosomes which appear to have formed a metaphase bivalent. Free fragments are very rare, always with a tapering strand, and in many cases clearly originating as a result of difficulty of separation. A bridge and fragment were found in one out of 100 late-anaphase cells. Second anaphase

is very regular; no marked erosion and constrictions or attached fragments were found. A bridge was observed in one out of 100 dyads. The plants in which meiosis was investigated had approximately 95 percent seed-set.

B. pseudolaevipes has seven pairs of large chromosomes, apparently similar in size to the seven pairs of large chromosomes in the *B. marginatus* complement (STEBBINS and TOBGY 1944). There are generally two chiasmata per bivalent at metaphase, and no appreciable variation in the diameter of the chromosomes (fig. 4). No bridge was found in 250 first anaphase cells; one free spherical fragment was found. In about nine percent of the mid-anaphase cells one chromosome pair exhibited difficulty of separation. About four percent of the late-anaphase dyads contained a lagging chromosome broken at the centromere or one or two free chromosome arms.

MEIOSIS IN THE HYBRID

Metaphase

Fourteen large chromosomes (seven from each parent) may be identified; however, none of these or any of the remaining 21 small chromosomes can be distinguished from one another. Univalents and bivalents were the only configurations observed; the bivalents were generally oriented at the equator and the univalents distributed at random.

The following data on chromosome pairing were compiled from 24 cells. There is an average of approximately seven, and a range of five to 12 bivalents per cell. The majority of these are made up of the large chromosomes of the *marginatus* and *pseudolaevipes* complements (fig. 9, a–g), but an occasional bivalent consists of two small chromosomes from the *marginatus* set (figs. 6, b; 9, h, i) (also see STEBBINS 1947); there is an average of approximately 1.3 small bivalents per cell and a range of none to five per cell.

The number and distribution of chiasmata in each bivalent could be determined in only 17 cells; thus the following figures are approximate. The chiasma frequency averages 10.6 per cell, 1.6 per bivalent; the range is one to four chiasmata per bivalent. Two or more chiasmata are found in 41 of the 98 large chromosomes observed; 24 of these have two chiasmata in one paired arm. The small bivalents generally have only one chiasma, but may have as many as three. The terminalization coefficient for all bivalents is 0.32.

Two of the several hundred metaphase cells examined were clearly differ-

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FIGURE 2.—*B. marginatus*, metaphase cell with 28_{II}. × 900.

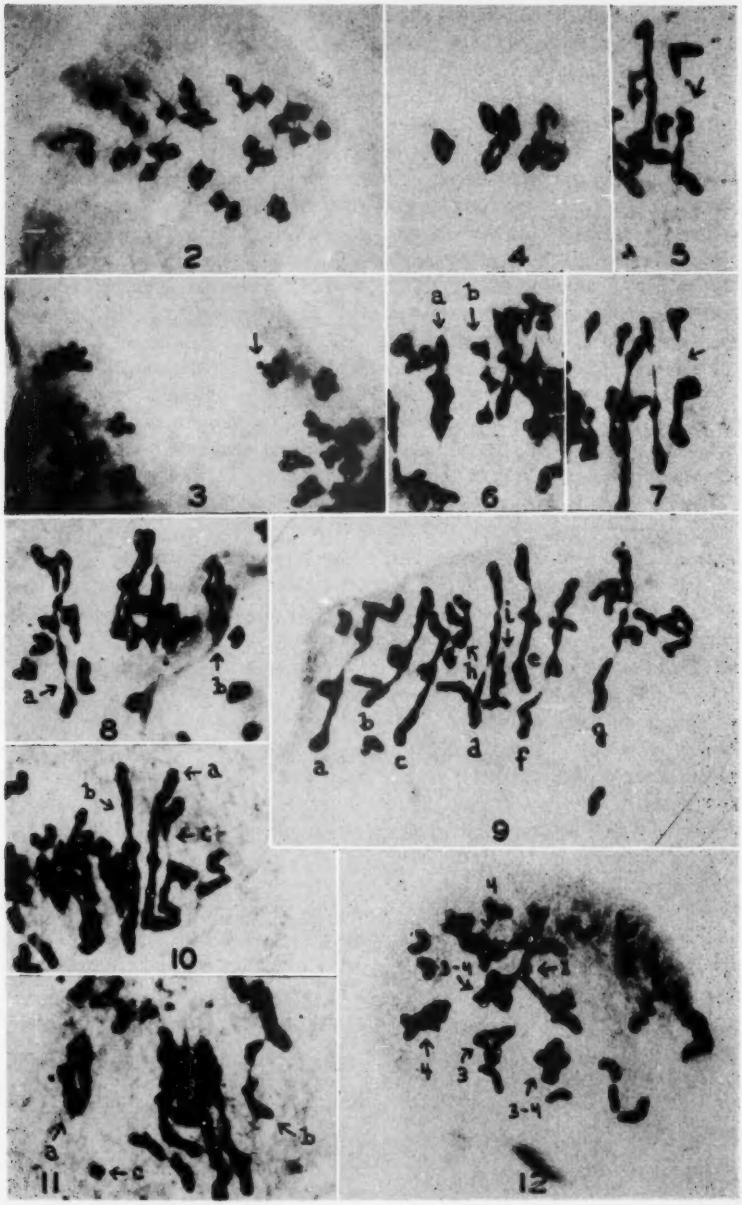
FIGURE 3.—*B. marginatus*, portion of anaphase cell with a single attached fragment. × 1275.

FIGURE 4.—*B. pseudolaevipes*, metaphase cell with 7_{II}. × 900.

FIGURES 5–8, 10–11.—Hybrid, portions of metaphase cells showing univalents and different types of bivalents. × 1275.

FIGURE 9.—Hybrid, metaphase cell with 7 large bivalents (a–g), 2 small bivalents (h–i), and 17 univalents. × 1275.

FIGURE 12.—Hybrid, atypical metaphase cell, the 6 bivalents not arranged on a plate; number of chiasmata per bivalent indicated. × 1275.


2
4
5
3
a
b
6
7
8
a
b
9
a
b
c
d
f
g
10
a
b
c
11
a
b
c
12
4
3-4
4
3
3-4

ent from the remainder in that the bivalents were not oriented on a plate, and the chiasma frequency was unusually high, averaging approximately three chiasmata per bivalent (fig. 12). There was only slight separation of centromeres in three of these bivalents, and little or no terminalization had taken place.

The most striking feature of metaphase in this hybrid is the occurrence in some bivalents of one or more "thin regions," that is, regions which are smaller in diameter than the remainder of the chromosome (figs. 5–11). This phenomenon is variable from chromosome to chromosome; a complete range is found from a slight decrease in chromosome thickness to a fine thread (fig. 10, a–c). The thin regions, although probably accentuated by smearing or pressure, are not produced in this manner, since bivalents of this type were also found in sectioned material.

Thin regions are found in both large and small bivalents, but never in univalent chromosomes. They are found only in the paired arms of a bivalent, always between the centromere and the chiasma (or first chiasma if there are more than one (fig. 10, c)), and generally, but not always, closely adjacent to the first chiasma. The part of the bivalent distal to a thin region ranges from a major portion of the arms to minute segments (figs. 14, 14 A). Generally the chromosome arms distal to the first chiasma appear to be unchanged in diameter (figs. 5–11). Thin regions occur in bivalents with chiasmata in only one arm and in bivalents with one or more chiasmata in each arm (figs. 6, a; 8, b), but appear to be more frequent in the former. A thin region may be present in only one chromosome of a bivalent (figs. 7; 11, b), or in each member of a bivalent (figs. 5; 6, b; 9, b–d), often appearing to be in the same approximate location in each arm relative to the chiasma (figs. 6, b; 9, b); two thin regions in a bivalent may be of equal or unequal thickness and a bivalent with an extremely thin region in one chromosome and a less markedly thin region in the paired arm of its partner is frequently found (figs. 9, a; 10, c). Generally there is only one thin region per chromosome arm, but occasionally two separate thin regions are found in one arm of a chromosome, or four in a bivalent (fig. 8, a).

The number of bivalents with one or more thin regions varies from cell to cell; in the cell shown in figure 9 one or two thin regions can be observed in eight of the bivalents, but in other cells only one or two bivalents may be of this type. It may be observed in figure 9 that thin regions are present in all seven of the large bivalents, whereas in other cells (fig. 11, a) there are one or more large bivalents which have no thin regions. Apparently the occurrence of thin regions in any chromosome may vary from cell to cell. No thin regions were found in either of the two exceptional metaphase cells described previously, which had numerous chiasmata and random orientation of bivalents (fig. 12).

In over 200 metaphase cells examined, only three were found to contain a single small fragment (fig. 11, c).

Anaphase

At the beginning of anaphase the two chromatids of each chromosome become distinct, but they still lie very close together. Fragments first appear at this stage (figs. 15, 18, 19).

At mid-anaphase the two chromatids of each chromosome have diverged except at the centromere. All members of bivalents have separated and some of the chromosomes have reached the poles; with one exception, no interstitial connection between separated chromosomes of a bivalent was observed. Generally the chromosomes which have reached the poles at this stage are the large chromosomes which were paired at metaphase, and those remaining at the equator are the small univalent chromosomes. The numbers of large and small chromosomes which lag at anaphase are as expected from the numbers of large and small univalents observed at metaphase.

Bridge frequency was determined in 150 cells at this stage, and approximately 20 percent of the cells contained a single bridge. It was possible to identify 22 of the 30 bridges observed as bivalent bridges (the chromosome at each end of the bridge consisting of two chromatids); no univalent bridges (with one chromatid at each end of the bridge) were found.

The fragments observed at anaphase in this hybrid are not numerous. About two-thirds of the cells contained one or more fragments; the maximum was seven per cell. The following types of free fragments are found: (1) *Free single fragments:* these are most frequently found, and range in size from very small spherical to rod-shaped bodies (figs. 17, d; 21, a). (2) *Paired free fragments:* these are very closely associated fragments of equal size, generally extremely small (fig. 13, b). (3) *Semi-paired free fragments:* two fragments of equal size which are separate but which lie fairly close together (fig. 13, a; 15); often one or both have a tapering strand, and the strands may point towards the same, but more often towards opposite poles.

The following types of attached fragments are found (fig. 1): (1) *Single attached fragment:* one fragment is attached by a strand to the end of a chromatid arm, the chromosome lying opposite exhibiting no comparable fragment (fig. 17, a). (2) *Single homologous attached fragments:* each one of two chromosomes, which lie opposite one another in the cell, has a single fragment attached to a corresponding arm; these fragments, which are of equal size, are attached either to matching chromatids or to opposite chromatids. (3) *Paired attached fragments:* two fragments of equal size are attached to the same end of a chromosome, each fragment attached to the end of one chromatid; the chromosome lying opposite this one in the cell has no attached fragments. (4) *Paired homologous attached fragments* (fig. 16, a): each of two chromosomes lying opposite one another in the cell has a pair of attached fragments, equal or approximately equal in size, attached to corresponding chromatids; these occur rarely. Another configuration, observed only once, consists of three homologous attached fragments (fig. 20, a, a').

It has been observed that attached fragments are found on those chromo-

somes which have reached the poles at anaphase (or which in very early anaphase are plainly moving towards the poles), and in many cases on similar chromosomes lying opposite one another. Attached fragments are generally found on large chromosomes (figs. 17, c; 18; 20, a, a′), only occasionally on small chromosomes (fig. 16, b), and thus agree with the relative frequency of large and small bivalents at metaphase. These fragments are found on both long and short arms of chromosomes; in either case the chromosome arms bearing attached fragments are always oriented in relation to the equator as if they had been the paired arms in a bivalent (figs. 16–18, 20–21). (It is extremely doubtful that the appearance of fragments on paired arms of chromosomes can be attributed to tensions at anaphase separation of such paired arms, since in the related hybrid *B. Trinii* × *maritimus* attached fragments are frequently found on univalent chromosomes.) Thus the attached fragments observed in this hybrid appear to be formed by paired chromosomes and by the chiasma-forming arms of these chromosomes.

Minute spherical bodies are occasionally found in the position of attached fragments (fig. 24, c), generally but not always associated with small chromosomes, either at the poles or lagging. There is some question whether these minute bodies are an extreme in attached fragment size, or whether they are concerned with the special behavior of the ends of chromosomes to be described below.

Lagging chromosomes frequently divide prematurely at late first anaphase. Usually the two chromatids separate and each moves poleward in a normal manner, with the centromere leading (fig. 23, b). However, in the majority of the late anaphase cells, one to several lagging and prematurely divided chromosomes exhibit atypical centromere behavior and movement of the

FIGURE 13.—Hybrid, anaphase cell; a = semi-paired free fragments; b = minute paired free fragments. × 1275.

FIGURE 14.—Hybrid, very early anaphase cell; arrow indicates minute knob distal to two thin regions of a bivalent. × 1275.

FIGURE 14A.—Outlined portion of cell in figure 14, showing double structure of thin region and knob, the knob probably consisting of four chromatids. × 2350.

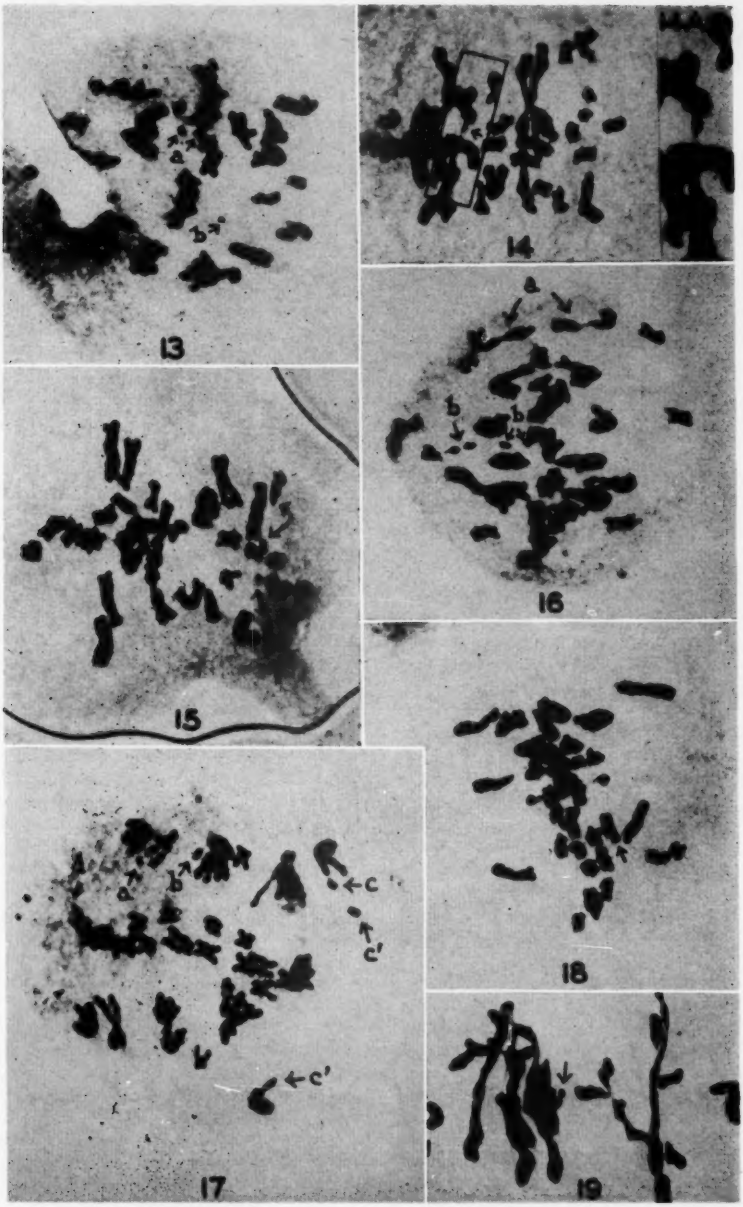
FIGURE 15.—Hybrid, very early anaphase cell; arrows indicate semi-paired free fragments and the small chromosomes from which they have arisen. × 1275.

FIGURE 16.—Hybrid, early anaphase cell; a = large chromosome with paired homologous attached fragments on the long arms; b = 3 free fragments and one attached fragment, formerly paired homologous attached fragments of small chromosomes. × 1275.

FIGURE 17.—Hybrid, anaphase cell; a = single attached fragment on the long arm of a large chromosome, the large chromosome opposite is normal; b = fragment attached to the short arm of a large chromosome; c = large chromosome with attached fragment on one long arm, c′ = free fragment of the same size, and large chromosome with deleted long arm (formerly single homologous attached fragments); d = single free fragment. × 1275.

FIGURE 18.—Hybrid, very early anaphase cell; arrow indicates single free fragment and large chromosome with one deleted arm, the large chromosome lying opposite is normal. × 1275.

FIGURE 19.—Hybrid, portion of very early anaphase cell; arrow indicates fragment separating from large bivalent. × 1275.


13
14
15
16
17
18
19
a
b
c
c'

chromatid ends toward the poles. This phenomenon is exhibited principally by the small chromosomes, but occasionally by a large lagging chromosome (fig. 26, gg). Note that in some cases the chromosome has reached the pole, preceded by one chromatid end (fig. 26, dd, ee).

Occasionally the two chromatids of a lagging chromosome have separated completely, and each appears to move end first towards the pole (fig. 23, a′). More often the two chromatids have moved apart only a little, apparently being held together by matrix connections, and one or more of the chromatid ends may be drawn out in the direction of the poles (figs. 22, c; 24, d; 26). Connections are observed between all parts of sister chromatids, but most frequently between centromeres or regions adjacent to centromeres. Sometimes the connecting strands between sister chromatids have broken and the chromatids are completely separate, with only indications of their previous association (fig. 26, y).

The ends of the chromatid arms which are oriented towards the poles may be round or very attenuated, and all intermediate types are found. All four ends of a prematurely divided chromosome may be oriented in the direction of the poles (fig. 26, a–d, x) but no chromosome was found with more than two ends strongly attenuated towards the poles. Most commonly the ends of the two long or two short arms exhibit this behavior (fig. 26, k–r), less often only one arm-end is drawn towards the pole (fig. 26, h–j). Occasionally one

FIGURE 20.—Hybrid, anaphase cell; a = large chromosome with paired attached fragments on the short arm; a′ = homologous large chromosome with an attached fragment of the same size on one short arm. × 1275.

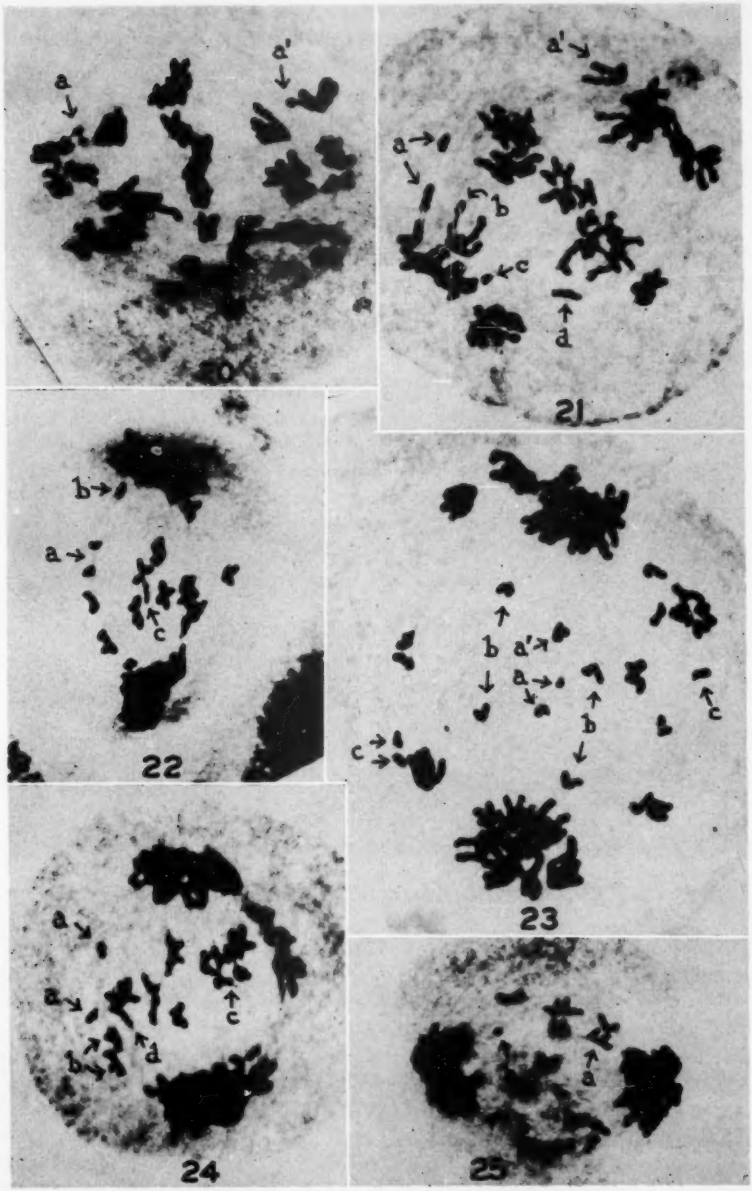
FIGURE 21.—Hybrid, anaphase cell; a = large chromosome with one fragment attached to a long arm, the other long arm with a similar strand, but the fragment has broken away (formerly paired attached fragments); a′ = the large chromosome lying opposite is normal; b = large chromosome with one attached fragment on a long arm; c = small single free fragment; d = large rod-shaped fragment, probably a whole arm of a small chromosome; note eroded appearance of the chromosomes. × 1275.

FIGURE 22.—Hybrid, late anaphase cell with several lagging small chromosomes; a = two separate long arms of a small chromosome with faint connecting strand; b = two closely associated short arms of a small chromosome; c = prematurely divided lagging small chromosome, the end of the lower chromatid attenuated towards the lower pole (see fig. 26, j). × 1275.

FIGURE 23.—Hybrid, late anaphase cell; a,a′ = prematurely divided and separated chromatids of a small chromosome, the ends oriented towards the poles, a with short and long arm separated; b = prematurely divided and separated chromatids of two small chromosomes moving normally to the poles with centromeres leading (compare with a′); c = separate long arms of small chromosomes. × 1275.

FIGURE 24.—Hybrid, late anaphase cell with numerous small lagging chromosomes; a = separate arms of small chromosomes; b = two small chromosomes, each lacking one chromatid arm; c = prematurely divided small lagging chromosome with a granule attached to the end of each chromatid; d = prematurely divided small lagging chromosome, the end of one chromatid attenuated toward the lower pole. × 1275.

FIGURE 25.—Hybrid, late anaphase cell with several small lagging chromosomes; a = small prematurely divided lagging chromosome, the long arms of the chromatids held together adjacent to the centromere; b = the separated short arm of this chromosome. × 1275.


a
a'
20
a'
a
b
c
d
21
b
a
c
22
b
a'
a
b
c
c
23
a
a
c
b
d
24
a
25

chromatid appears to be stationary and one or both ends of the attached sister chromatid are attenuated towards the pole (fig. 26, h). With rare exceptions the sister chromatids appear to move towards opposite poles; figure 26, t–v, shows both long arms of a divided chromosome attenuated in the direction of the same pole.

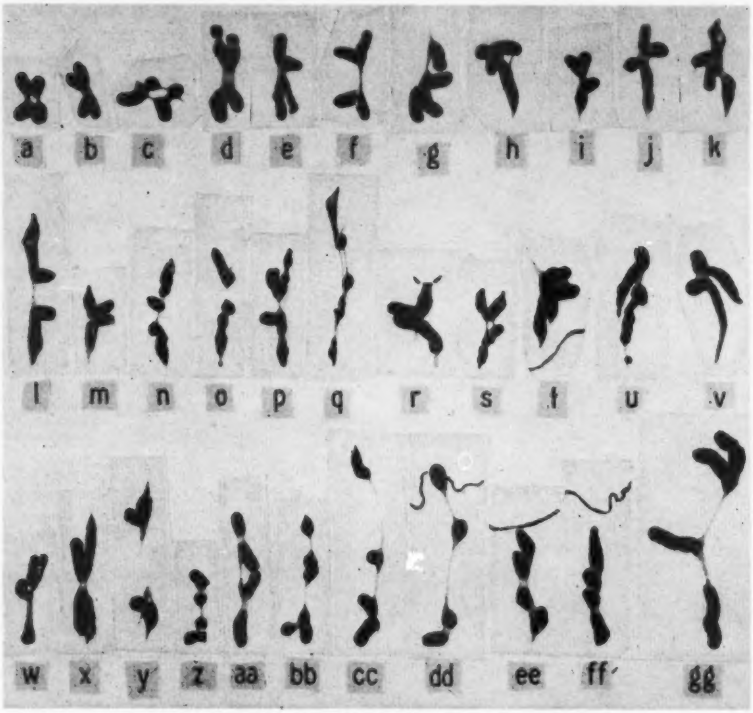


FIGURE 26.—Hybrid chromosomes at first anaphase; the upper and lower sides of the figure represent the direction of the poles; the dotted line in r indicates an overlying chromosome; the full lines in dd, ee, and ff indicate the edges of the polar groups in these cells. × 2380.

It is frequently observed that the two arms of a free or attached chromatid are separated by a short lighter staining region, or more widely by a strand which may, in extreme cases, be longer than the chromosome arm (fig. 26, z–gg). These attenuated centromere regions were found only in lagging chromosomes, thus almost exclusively in small chromosomes but occasionally in a lagging large chromosome (fig. 26, gg).

Occasionally one or more free chromosome arms are found in a late anaphase cell (fig. 21, d; 23, c). Sometimes one arm has broken away from a

lagging chromosome (figs. 24, a, b; 25, a, b), or the arms of a single free chromatid have separated (fig. 23, a). Only one lagging chromosome was observed which appeared to be dividing transversely in such a way as to separate both short arms from both long arms; however, several cells were found in which the results of such transverse division could be seen (fig. 22, a, b). It could not be determined whether individual free chromosome arms were telocentric or acentric, whether the transverse break occurred in the centromere ("centromere misdivision," DARLINGTON 1939), or immediately adjacent to the centromere.

Second Division

Fifty early-mid-anaphase cells were studied and no attached fragments were found; occasional free single or paired fragments are observed. Nine percent of the 100 mid-late-anaphase dyads observed contained one bridge, a frequency not inconsistent with the number and location of chiasmata at metaphase.

An occasional lagging chromosome is found at late anaphase in which the centromere region is drawn out into a fine thread, and free chromosome arms are occasionally found.

The connections observed at first anaphase between separated chromatids of prematurely divided chromosomes do not appear in chromosomes dividing at second anaphase. Some chromosome ends are slightly pointed, but no striking attenuations of chromosome ends towards the poles are found. However, some of the lagging chromosomes appear to be oriented at random, and occasionally the end of a chromosome arm precedes the remainder of the chromosome to the pole.

DISCUSSION

There are certain similarities in meiosis of *B. marginatus* and the *marginatus* × *pseudolaevipes* hybrid, namely attached fragments, difficulty of separation of bivalents, and chromosome erosion at anaphase; however, difficulty of separation and chromosome erosion occur more frequently in the parent and fragments occur much more often in the hybrid. The hybrid exhibits metaphase bivalents with thin regions, which are not found in either parent, as well as several types of fragments not observed in *marginatus,* namely free semi-paired and paired fragments, and attached paired and paired homologous fragments. These differences in meiosis observed in the hybrid are attributed to interaction of the genomes of *marginatus* and *pseudolaevipes.*

Spontaneous chromosome breakage, apparently similar to that induced by irradiation, has been reported to occur in meiotic first anaphase in two materials in which bivalent and univalent chromosomes are present at metaphase. In Kinugasa (HAGA 1937) both bivalent and univalent chromosomes undergo breakage. In the hybrid *Bromus Trinii* × *maritimus* (WALTERS 1950), which has an approximate average of 4 bivalents and 31 univalents per cell, breakage occurs principally in the univalents; many fragments attached to univalent

chromosomes and some univalent bridges are observed at first anaphase. However, the present hybrid differs from these in that, although an approximate average of seven bivalents and 21 univalents per cell is found at metaphase, no univalent bridges are observed at first anaphase, nor are fragments attached to univalent chromosomes: breakage here appears to be restricted to the paired chromosomes, and further to the chiasma-forming arms of these chromosomes. The fragments observed in the *B. marginatus* × *pseudolaevipes* hybrid cannot, then, be attributed to chromosome breakage of the type described previously.

Fragments attached to separating bivalent chromosomes have occasionally been observed in other genera, namely in Lilium (RICHARDSON 1936; HAGA 1943), Secale (MÜNTZING and PRAKKEN 1941), and Fritillaria (DARLINGTON 1939) and attributed to tensions arising at anaphase between centromeres and immovable regions of chromatids. Such hypotheses, however, probably imply delayed separation of members of affected bivalents and the frequent occurrence of interstitial connections between them, whereas in this hybrid chromosomes with attached fragments may separate from one another at anaphase as rapidly as chromosomes without attached fragments, often reaching the poles at early anaphase, and separating chromosomes with an interstitial connection are very rare.

An hypothesis to explain the formation, previous to anaphase, of fragments related to paired chromosomes and to their chiasma-forming arms has been suggested to the author by DR. BARBARA MCCLINTOCK. It is known that fragments produced by irradiation in early prophase of meiosis may not be visible until anaphase (GOODSPEED 1929), and that an acentric fragment may be connected by a matrix strand to the end of a chromatid at anaphase (MCCLINTOCK 1938). It is then suggested that the fragments described above may result from cross-over breaks in paired chromatids at early prophase, which are not followed by reunions of broken ends to produce cross-over chromatids. Later in prophase matrix accumulates around the paired chromosomes so that these form a bivalent, and paired centric chromatids and acentric fragments are held together at the region of cross-over breakage by the matrix until anaphase. Thus a "chiasma" observed at metaphase may represent the point of crossing-over of two chromatids or a region where cross-over breakage has not been followed by cross-over reunions. At anaphase the two chromatids of each chromosome separate, except at the centromere, and the movement of chromosomes and chromatids dislodges the acentric fragments. An attached fragment at anaphase, then, represents an acentric fragment connected to the end of the broken centric chromatid by a matrical strand.

Crossing-over may take place on either side of the breakage regions, and in certain cases may influence the type of fragment-configuration observed at anaphase. It is assumed that restitution of broken ends sometimes takes place, producing the single attached fragment from two cross-over breaks or three homologous attached fragments from four cross-over breaks. Reverse reunion of paired chromatids may occasionally take place, producing a bridge

and fragment at anaphase. MATSUURA (1946) reports that in Trillium a single bivalent bridge is frequently formed by 4-chromatid breaks within a chiasma region.

If a single chiasma in a metaphase bivalent is formed by cross-over breaks, or if cross-over breaks are present proximal to a true chiasma, poleward movement of the centromeres at metaphase may separate the centric and acentric chromatid segments, the two parts of the chromatid being connected only by a matrical strand; thus one or more thin regions may be produced in a metaphase bivalent. An acentric fragment distal to a chiasma should not be subject to tension and thus no matrix connection (*i.e.*, thin region) should be visible at metaphase.

Various types of cross-over breaks and reunions, and the attached fragments which result from them are shown in figure 1.

Free fragments arise at early anaphase, when acentric segments which are not connected to the remainder of the chromatid by matrix strands are dislodged, or later at anaphase by means of breakage of the connecting strands of attached fragments. Free single fragments, which are found most frequently, may arise from any of the several types of attached fragments: as shown in figure 17, c, c′ from single homologous attached fragments and in figure 21, a, a′ from paired attached fragments. Semi-paired free fragments with strands pointing towards the same pole probably arise from paired attached fragments (fig. 16, b), those with strands pointing towards opposite poles from single homologous attached fragments. Because sister chromatid arms are separated at mid-anaphase, paired free fragments should not arise at this stage, but are probably freed at very early anaphase.

The hypothesis that fragments arise from cross-over breaks accounts satisfactorily for all the types of fragments observed and, since the regions of cross-over breaks simulate chiasmata, for the observed association of fragments with chiasma-forming arms of bivalents. The absence of attached fragments at second anaphase indicates that all matrix connections have broken previously to this stage.

Many more thin regions are found in bivalents at metaphase than attached or free fragments at anaphase, suggesting that the majority of these thin regions are produced independently of fragment formation. Since the thin regions observed at metaphase in this hybrid are never found in univalents or in unpaired arms of bivalent chromosomes, their formation appears to depend upon chromosome pairing and upon the presence of a chiasma in the affected chromosome arms. The formation of these thin regions seems further to be dependent upon tensions between poleward moving centromeres of bivalents at metaphase and an interstitial chiasma, as suggested by DARLINGTON (1937), since thin regions are never found distal to the first chiasma, and were not observed in the non-oriented bivalents in exceptional cells in which the spindle was apparently inactive. The diverse thin regions in the present hybrid are assumed to be the result of various degrees of uncoiling of the major spiral of the chromonema. Although thin regions frequently occur just proximal to the first chiasma, the occasional observation of a thin region in

only one member of a bivalent, and of a thin region not immediately adjacent to the chiasma suggests the possibility of a localized tendency to uncoil. The chromonema is apparently able to recoil when the tension between the centromere and the first chiasma is removed at anaphase, although irregularity in recoiling may account for the variation observed at anaphase in chromatid diameter and for the presence of certain constrictions.

Metaphase bivalents with thin regions, as in the present hybrid, are found in *B. Trinii* × *maritimus* (WALTERS 1950) and *B. Trinii* × *marginatus* (WALTERS, unpub.); however, none of the parent species exhibit such bivalents, nor do the hybrids *B. maritimus* × *marginatus* and *B. carinatus* × *marginatus* (WALTERS, unpub.). Thus the causative factors are variable among related hybrids. The variation in the occurrence and extent of thin regions from bivalent to bivalent and from cell to cell in the present hybrid indicates that the causative factors are also variable in and among cells. These facts point away from arrest of terminalization at non-homologous segments or spindle elongation as responsible factors, since the former should occur in related hybrids and the latter should affect all bivalents in a cell. It is then suggested that thin regions may be produced by an increase in strength of movement of centromeres towards the poles in certain hybrids and that variation in strength of centromere movement may occur among individual cells and bivalents in these hybrids.

It has been observed in this hybrid that "fragments" may consist of part or all of the chromosome arm; thus centromere breakage (*i.e.*, breakage in or adjacent to the centromere) occurs with the general chromosome breakage discussed above, and the question arises as to whether these are indeed separate phenomena. However, non-centromere breakage generally occurs in early- and mid-anaphase, primarily in large paired chromosomes which are moving polewards, but centromere breakage is generally observed at late-anaphase, principally in the small lagging univalent chromosomes. The occasional occurrence of centromere breakage in a large lagging chromosome indicates that this is not a special property of the small *marginatus* chromosomes, but of any chromosome which lags at first anaphase. It is therefore believed that the centromere breakage observed in this hybrid occurs independently of the general chromosome breakage, and may be comparable with that occurring in lagging chromosomes in many materials which do not exhibit any other chromosome breakage.

Some chromosomes which lag and divide prematurely at first anaphase exhibit non-centromeric movement towards the poles, and in some cases these are observed to reach the poles, preceded by one chromatid end. Further, the ends of such chromatids may be attenuated in the direction of the poles. There are suggestions in other materials of non-centromeric chromosome movement at anaphase by spindle elongation (RIS 1949, ÖSTERGREN 1949), or by cytoplasmic currents (CARLSON 1938). KOLLER (1938) has observed in an asynaptic Pisum univalents which resemble the less extreme types with atypical movement found in this hybrid (fig. 26, a–c), and suggests that these may possibly be due to stretching of the spindle. SANCHEZ-MONGE (1950) has ob-

served non-centromeric movement of univalents at first and second anaphase in Triticum, accompanied by breakage of the centromere region, and has attributed this to separation of the arms of the chromosome by spindle forces or cytoplasmic currents following failure of attachment of spindle fibers to centromeric chromomeres.

It has been observed in the *B. marginatus* × *pseudolaevipes* hybrid that non-centromeric movement is exhibited principally by the small lagging univalent chromosomes. It may then be suggested that in this hybrid the centromeres of certain lagging univalents are ineffectual or inactive, and that the chromosomes are carried passively towards the poles by external forces such as spindle elongation or cytoplasmic currents. Movement of two arms of a chromatid towards opposite poles (or of two attached chromatids towards opposite poles) may produce attenuated centromeres (or regions adjacent), which may occasionally break.

There are some indications that the ends of attached chromatids lying in the longitudinal axis of the spindle may be attenuated because of their resistance to movement by external forces. It may be noted that no attenuated chromatid ends were observed at second anaphase, at which stage separated sister chromatids are not attached to one another by strands, as at first anaphase. Furthermore, chromatids with strongly attenuated ends generally do not exhibit stretched centromere regions which, if present, would be expected to decrease markedly the tensile effects of the spindle forces on the chromatid ends (fig. 26, h–v). However, there are also indications that the attenuation of chromatid ends is determined autonomously. Occasionally ends of sister chromatids are attenuated towards the same pole; presumably two chromatids moving in the same direction would offer little resistance to movement. Also, it has occasionally been observed that one chromatid exhibits end-movement and attenuation, and its attached sister chromatid is unaffected. Furthermore, it has been observed that no more than two chromatid arms of any chromosome are strongly attenuated.

Terminal secondary centromeric regions which move the ends of bivalent chromosomes polewards have been observed in Zea (RHOADES and VILKOMERSON 1942) and in Secale (ÖSTERGREN and PRAKKEN 1946). It is believed that the present hybrid represents a third example of chromosome movement by centromeric action of chromatid ends. In this case it is suggested that in these Bromus species both the centromeres and the terminal regions of chromosomes have the property of movement towards the poles, although this function is secondary and imperfect in the terminal regions. In the hybrid the centromeres of some univalent chromosomes may be inactivated and the terminal regions are able to function so that the chromosome moves towards the pole and in some cases reaches the pole.

SUMMARY

B. pseudolaevipes (2n = 14) exhibits completely regular meiotic divisions. *B. marginatus* (2n = 56), however, occasionally exhibits certain meiotic irregularities which are also found in the hybrid, namely some stickiness, chro-

mosome erosion, and occasional attached fragments. The metaphase chromosomes of both species are relatively uniform in diameter.

The hybrid *B. marginatus* × *pseudolaevipes* (2n = 35) exhibits an approximate average of seven bivalents and 21 univalents per cell at first metaphase. Free fragments are very rare at this stage. One or both chromosomes of a metaphase bivalent may exhibit a "thin region," that is, a region between the centromere and first interstitial chiasma which varies in diameter from slightly less than that of the free arm of the chromosome to a fine thread; the arms distal to the first chiasma are unaffected. The number of bivalents of this type varies from cell to cell. It is believed that the thin regions are the result of various degrees of uncoiling of the major spiral of the chromonema, produced by tension between poleward moving centromeres and interstitial chiasmata. It is suggested that the strength of centromere movement at metaphase may have increased in this hybrid, and that it may vary in individual bivalents.

Free and attached single and paired fragments are found at anaphase; single and paired fragments are sometimes attached to corresponding arms of homologous chromosomes. The chromosome breakage observed here differs from that previously reported in that fragments are found to be attached only to chromosomes which have been paired and to arms which have formed chiasmata. It is suggested that these fragments may be produced by cross-over breaks in paired chromatids at early prophase which do not reunite to form cross-over chromatids; restitution of one or more chromatids may sometimes occur. The matrical strand connecting the attached acentric fragment to the end of the broken centric chromatid sometimes breaks, producing a free fragment.

Centromere breakage, which is observed at first anaphase, is believed to occur independently of the other chromosome breakage.

Non-centromeric chromosome movement is observed, principally in some lagging univalents which divide prematurely at first anaphase, but whose chromatids remain connected by strands: one or more chromatid ends appear to move towards the poles, and sometimes the chromosome reaches the pole end-first. It is suggested that the centromeres of such chromosomes may be inactive, movement taking place by means of spindle forces or cytoplasmic currents. Similar chromosomes with ends attenuated towards the poles indicate that in these species the terminal regions of the chromosomes may have a secondary and imperfect centromeric capacity, which may be expressed if the centromere does not function.

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RELATION BETWEEN STERILITY AND MORPHOLOGICAL CHARACTERS IN AN INTERSPECIFIC NICOTIANA CROSS[1]

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SPECIES with genetic systems sufficiently similar to permit hybridization and production of abundant progeny in subsequent generations, yet sufficiently discrete to give partial sterility and wide phenotypic diversity among hybrid segregants, present favorable material for studying two problems in evolution, namely: (1) the relation between genetic isolation and morphological divergence, (2) the relation between gamete elimination and restrictions to recombination. If hereditary factors determining hybrid sterility are independent of those controlling different morphologies, no correlation between the two would be expected. Conversely, if the genes which have been accumulated in each species to produce the different phenotypes are those which in hybrid combination cause sterility, then a correlation of higher sterility with recombination types would be expected in segregating generations. Structural chromosomal differences may also cause sterility in hybrids and this effect may be combined with genic sterility. When the structural differences are so small as not to materially influence chromosome pairing, "cryptic structural hybridity" (STEBBINS 1945), the two causes are usually not distinguishable without further tests (STEPHENS 1950).

The subject of interrelationships between isolating mechanisms and interspecific differences has been reviewed recently by STEBBINS (1950). It is the purpose of this paper to contribute information on the problem by presenting an analysis of crosses between two species of Nicotiana which are favorable materials for study.

MATERIALS AND METHODS

Inbred strains of *Nicotiana Langsdorffii* Weinm. (n = 9) and *Nicotiana* × *Sanderae* Hort. var. Sutton's Scarlet (n = 9) were used as parental species. The former is a valid taxonomic species native to southeastern Brazil and adjacent areas. The latter is a "commercially adaptive" introgressive product of corolla color genes from *N. Forgetiana* ex Hemsl. (n = 9) in *N. alata* Lk. and Otto. (n = 9). The gross chromosome morphology in *N. alata* and in the strain of *N. Sanderae* used here is similar (ABRAHAM 1948). The cross between *Langsdorffii* and *Sanderae* has succeeded only when *Langsdorffii* is used as the female parent (SMITH 1937a). The F_1 hybrid is intermediate in most plant characters but exhibits marked heterosis in general plant growth.

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Pollen sterility

The interspecific F_1 has 50–55 percent aborted pollen while the parent species usually have less than 15 percent. The most frequent chromosomal associations found at meiosis in the F_1 are one quadrivalent, six bivalents and two univalents. Irregular distribution of chromosomes due to non-bivalent pairings undoubtedly accounts for much of the sterility in the F_1. The amphidiploid is a segmental allopolyploid (STEBBINS 1950); lagging univalents are observed at the first meiotic anaphase, and the plants segregate upon selfing so that other than homogenetic pairings evidently take place. The pollen is 20–25 percent sterile. A comparison of gamete sterilities between F_1 and amphidiploid is generally considered to be a means of distinguishing between genic and chromosomal sterility; however, when the latter is a segmental allopolyploid this criterion is not conclusive, for irregularities in distribution of chromosomal materials may contribute to the sterility of the amphidiploid.

The F_1 hybrid also has reduced fertility of female gametes as evidenced from a count of the number of seeds formed in the capsules. With plants grown under the same greenhouse conditions the average number of seeds per capsule w 368 in *N. Sanderae*, 365 in *N. Langsdorffii* and 110 in the F_1. In spite of the lower number in the F_1 there is little, if any, reduction in the reproductive potential per F_1 plant since, as a consequence of hybrid vigor, many more capsules are formed.

The percentage of pollen sterility was determined in the F_1, F_2 and first backcrosses to each parent species. The technique used was to dust pollen from recently dehisced anthers into a drop of propiono-orcein and to examine the preparation under a microscope at 200× magnification. Shriveled, unstained grains were tallied as abortive and well-filled, stained grains as functional. Spot checks were made in representative areas of the slide and usually 300 to 500 grains were counted for each determination.

The question as to whether the stainable grains of each generation were equally capable of germinating was investigated by growing pollen of *N. Sanderae* and of the F_1 on an artificial medium (BISHOP 1949). Two hours after sowing the cultures were fixed and stained and the percentage of normal, filled grains that had germinated was calculated on the basis of about 1500 observations (see fig. 1). Twenty-nine percent of the stained *Sanderae* pollen had germinated by this time and thirty-six percent of the stained F_1 pollen. *N. Langsdorffii* is more rapid in plant development than *N. Sanderae* and the presence of germplasm from this species in the F_1 may have caused the higher percentage. It seems clear that at least as high a proportion of stainable pollen in the F_1 (which contains genetic recombinations) is viable, as in the parental species (all pollen of which has the same genotype). Therefore, the percentage of pollen abortion is evidently an adequate criterion of the amount of male gamete elimination, with the possible exception of differential selective phenomena that may occur between pollen germination and fertilization.

Generally there is no difficulty in classifying pollen grains as viable or non-viable on the basis of staining reaction. However, pollen may be scarce and clumped but not contain a high percentage of aborted grains. In these

cases, which were rare and not included in the analysis, the amount of aborted pollen observed did not give a significant estimate of male gamete elimination.

Maximum chromosomal sterility might be expected among gametes of the F_1, since there is a maximum of structural differences among the two sets of chromosomes in this generation. Maximum genic sterility is found in certain genetic recombinations of the F_2 and first backcross generations as evidenced by the occurrence of segregants with over 50 and up to 86 percent pollen abortion (table 2). The distribution of percentage of pollen sterility forms a continuous series in segregating generations and is considered to be governed by multiple factors.

Male sterile plants occur in the F_2 and $F_1 \times$ *Sanderae*. They constitute a distinct sterility category in that no pollen grains, empty or filled, are formed. EAST (1932) interpreted male sterility in this cross as due to an interaction

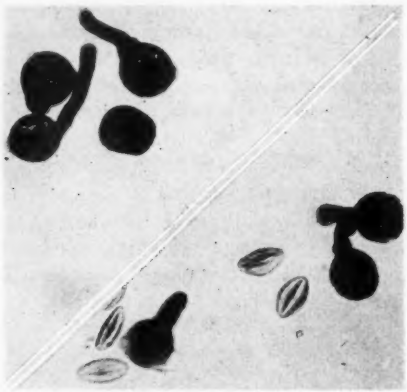

FIGURE 1.—Germinating pollen grains of *N. Sanderae*, left, and F_1 *N. Langsdorffii* × *N. Sanderae*, right, two hours after sowing on an artificial medium. Magnification × 300.

of alleles at the self-incompatibility locus (S) with cytoplasm of *N. Langsdorffii*. He stated, " Plants carrying two S factors other than S_f (from *Langsdorffii*) were male sterile in the presence of cytoplasm derived largely from *N. Langsdorffii*." The writer's observations confirm this hypothesis to the extent that no male sterile plants were found in the crosses *Langsdorffii* $\times F_1$, $F_1 \times$ *Langsdorffii*, or *Sanderae* $\times F_1$. Male steriles were not included in the analysis presented in this paper since the genetic phenomenon involved is apparently distinct from that determining the continuous distribution in amount of pollen abortion otherwise observed.

The strain of *N. Sanderae* var. Sutton's Scarlet used in these experiments is self-compatible.

Morphological differences

The most pronounced and easily measured morphological differences between *N. Sanderae* and *N. Langsdorffii* are the size and shape of the corolla. The corolla differences between the two species have been described and illustrated in previous publications (SMITH 1937b, 1943, 1950). *N. Sanderae* has

a long corolla tube (due primarily to elongation of the basal portion, the part proximal to the insertion point of the stamens), long limb, and deep lobing; whereas *N. Langsdorffii* has a short tube base, short limb, and no lobing. The three measurements used, therefore, were length of the tube base, length of the longest limb, and degree of lobing (as measured by the length of the longest limb minus the length of the adjacent sinus).

GENETIC ANALYSIS OF MORPHOLOGICAL DIFFERENCES

The corolla characteristics studied show continuous variation in segregating populations and there is ample evidence that their inheritance is controlled by multiple genetic factors that are well distributed throughout the specific complements (SMITH 1937b). As a consequence of multifactorial inheritance the range of segregation is restricted in both the direction of recombinations and parental combinations (ANDERSON 1939; DEMPSTER 1949). The magnitude of this general restriction is dependent on the number of independent segregating units. Linkage, which would be expected to occur in characters of this type which are governed by many genes on relatively few chromosomes, acts to increase the variance in the direction of parental combinations (ANDERSON 1949; DEMPSTER 1949). Similar developmental patterns for the corolla in the two species restricts the variance in the direction of new recombinations of parental characteristics (SMITH 1950). The net result of these three properties is to cause the distribution pattern of the F_2 to be in the form of an ellipse which is restricted throughout due to multiple factor inheritance, elongated in the direction of the parents due to linkage, and narrowed in the direction of recombinations due to developmental restrictions.

TABLE 1

Distribution of corolla measurements for the tube base, limb and lobing into three size classes, namely: L (most Langsdorffii-like), S (most Sanderae-like) and M (intermediate).

	Number of individuals			
	L	M	S	Total
F_2 generation				
Tube base	110	106	108	324
Limb	109	110	105	324
Lobing	105	120	99	324
Total	324	336	312	
F_1 × *Langsdorffii*				
Tube base	39	41	36	116
Limb	44	29	43	116
Lobing	41	39	36	116
Total	124	109	115	
F_1 × *Sanderae*				
Tube base	42	45	36	123
Limb	47	31	45	123
Lobing	41	41	41	123
Total	130	117	122	

Another property of genetic systems, which if present would cause restriction of recombinations, is differential gamete elimination acting against recombinative genotypes (ANDERSON 1939). Experimental evidence both for and against the occurrence of this phenomenon has been cited by STEBBINS (1950). Data on the relation of gamete elimination (as measured by pollen abortion) and recombination of morphological differences (as measured by corolla characteristics) is presented in this paper.

In order to investigate these relationships the F_2 and first backcross generations were divided into groups or blocks representative of different combina-

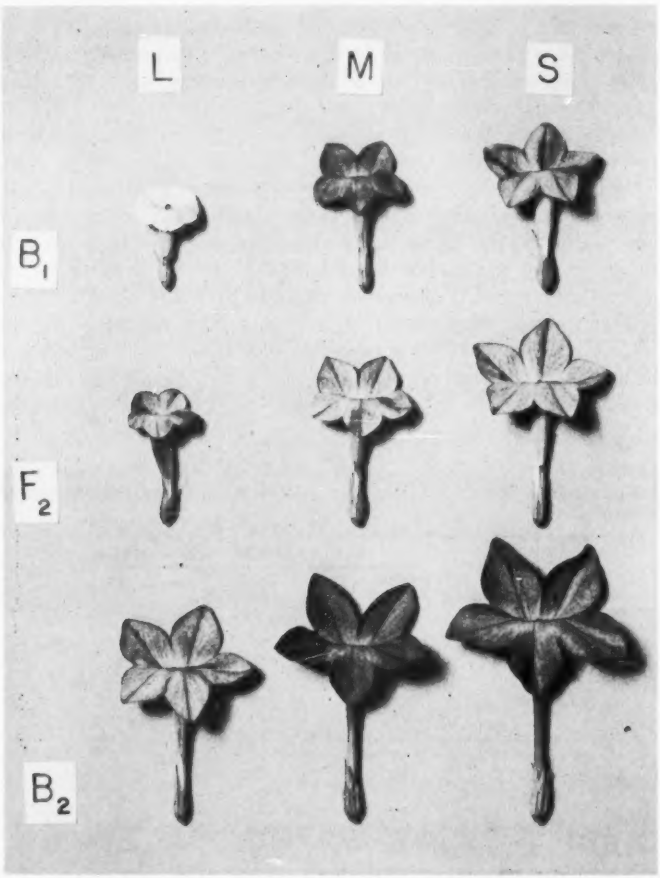

FIGURE 2.—Corollas (× ½) of the $F_1 \times$ *Langsdorffii* (B_1: top row), the F_2 (F_2: middle row), and the $F_1 \times$ *Sanderae* (B_2: bottom row). The corollas shown are representative for the group of segregants in each generation that are the most *Langsdorffii*-like (L: left column), intermediate (M: middle column) and most *Sanderae*-like (S: right column) for the three characters tube base, limb and lobing.

tions for the characters tube base, limb and lobing. Each of the three segregating generations was divided into three groups for each character (fig. 2), namely: those that were most *Sanderae*-like (S), those that were most *Langsdorffii*-like (L), and those that were intermediate (M). The distribution was arranged so that the number of individuals in each class was as nearly equal as possible (table 1).

Since each of the characters is evidently controlled in inheritance by many genes with cumulative effects, the groupings are roughly indicative of the

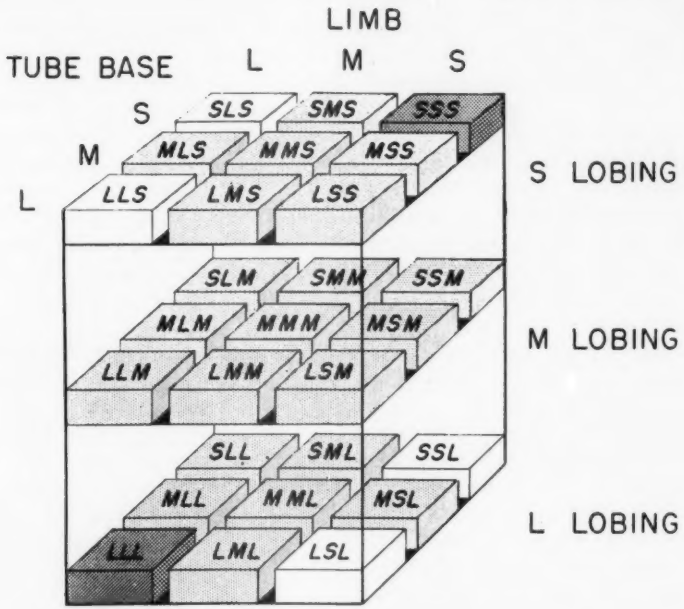

FIGURE 3.—Diagrammatic representation of recombination blocks formed by combining three size classes (S, M and L) for three quantitative characters (tube base, limb and lobing). The shading denotes three different groups of F_2 segregants according to their frequency of recovery. SSS and LLL, the most parental-like blocks, were recovered most frequently. The unshaded blocks—SLS, LLS, SSL, and LSL—were not recovered. The rest were recovered in intermediate frequencies (see table 2).

relative numbers of genes from each species (ANDERSON 1939; SMITH 1943). All possible combinations of the three classes for the three characters were made to form 27 different recombination blocks (fig. 3). These blocks are considered to constitute a reasonably sensitive criterion of different degrees of parental combination or hybrid recombination in the component segregants. For example, segregants classified in the L group for tube base, limb and lobing (LLL) undoubtedly possess the greatest preponderance of genes from *N. Langsdorffii;* while those classed as S for the three measurements (SSS phenotype) are undoubtedly largely of *Sanderae* genotype.

TABLE 2

Frequency of recovery and percentage of pollen abortion in types of recombinations for tube base, limb and lobing of the corolla in the F_2 and first backcross generations.

	Recombination types			F_2 Langs. × Sand.			F_1 × Langs.			F_1 × Sand.		
	TB	Limb	Lob.	Freq.	Pollen abortion (Av. %)	Range in % pollen abortion	Freq.	Pollen abortion (Av. %)	Range in % pollen abortion	Freq.	Pollen abortion (Av. %)	Range in % pollen abortion
1	L	L	L	64	37.1 ± 6.0*	2–86	28	41.2 ± 9.3	5–70	24	48.2 ± 10.8	15–77
2	S	S	S	52	27.3 ± 6.2	8–70	24	50.1 ± 10.2	35–65	21	23.7 ± 9.3	2–50
3	M	M	M	28	27.6 ± 8.4	8–52	13	43.8 ± 13.8	25–65	8	37.1 ± 17.1	1–70
4	S	M	M	26	33.2 ± 9.2	1–65	5	42.2 ± 22.1	37–50	9	22.4 ± 13.9	1–50
5	M	S	S	24	31.3 ± 9.5	10–70	7	47.1 ± 18.9	35–65	13	27.2 ± 12.2	5–65
6	M	L	L	18	35.3 ± 11.3	10–75	9	42.7 ± 16.5	10–64	10	42.3 ± 15.6	28–50
7	L	M	M	20	34.6 ± 10.6	15–50	5	43.0 ± 22.1	25–65	8	46.0 ± 17.6	20–66
8	S	S	M	13	31.3 ± 12.9	5–70	4	53.2 ± 25.0	37–66	1	40.0 ± 49.0	
9	M	M	S	9	29.9 ± 15.2	8–60	2	37.5 ± 34.2	30–45	1	31.0 ± 46.3	
10	M	M	L	7	30.0 ± 17.3	5–60	2	27.5 ± 31.6	20–35	1	55.0 ± 49.7	
11	L	L	M	7	24.4 ± 16.2	11–52	3	24.7 ± 24.9	12–37	3	18.3 ± 22.3	10–25
12	M	S	M	10	40.1 ± 15.5	10–60	6	52.8 ± 20.4	35–67	6	27.0 ± 18.1	10–60
13	L	M	L	9	44.8 ± 16.6	15–65	1	40.0 ± 49.0		2	50.0 ± 35.4	40–46
14	M	L	M	8	26.6 ± 15.6	10–50	2	33.5 ± 33.4	30–37	6	31.5 ± 19.0	20–45
15	S	M	S	5	34.0 ± 21.2	10–70	1	43.0 ± 49.5		1	3.0 ± 17.1	
16	L	S	S	6	26.3 ± 18.0	5–60	2	47.5 ± 35.3	45–50	4	35.0 ± 23.8	30–50
17	S	L	L	5	34.0 ± 21.2	10–58	1	35.0 ± 47.7		4	41.5 ± 24.6	30–51
18	S	L	M	6	43.7 ± 20.3	38–55	1	50.0 ± 50.0		0		
19	S	M	L	1	23.0 ± 42.1		0			0		
20	L	M	S	2	11.5 ± 22.5	10–13	0			1	1.0 ± 10.0	
21	L	S	M	2	38.0 ± 34.3	31–45	0			0		
22	M	L	S	1	60.0 ± 49.0		0			0		
23	M	S	L	1	25.0 ± 43.3		0			0		
24	S	S	L	0			0			0		
25	L	L	S	0			0			0		
26	S	L	S	0			0			0		
27	L	S	L	0			0			0		
Total				324		1–86	116		5–70	123		1–70

*Standard error calculated as $\sqrt{\frac{pq}{n}}$.

If the three characters were completely independent in inheritance and development the number of plants in each block would be approximately equal. The result obtained in the F_2 was that over one third of the segregants were included in the LLL and SSS blocks, those most parental-like (table 2). The characters are correlated as a consequence of both genetic linkage and correlated growth pattern. In addition to demonstrating a general correlation of the three corolla characters the frequencies in the blocks give further insight into the kinds of recombinations that occur. The next most frequent F_2 block is MMM which includes those segregants most like the F_1 in phenotype (table 2). Blocks 4, 5, 6 and 7 are next most frequent. They comprise those corollas with the same classes for limb and lobing, *i.e.*, LL, SS or MM and the tube base measurement is one class removed. For example, if the limb and lobe are S the tube base is M (not L). In blocks 8, 9, 10 and 11 the tube base and limb measurements are in the same class and the lobing measurement is one removed. In blocks 12, 13, 14 and 15 the tube base and lobing measurements are in the same class and the limb measurement is one class removed. Blocks 18 through 23 have three different classes combined. Blocks 24 through 27, which were not recovered, involve a combination of L with S for limb and lobing. The data on frequency of recovery of the different blocks demonstrate that there is a restriction to recombinations of the characters within the range of expression in the F_2. Restrictions in recombination of parental characteristics for this cross (SMITH 1950) and for the cross *N. Langsdorffii* × *N. alata* (ANDERSON 1939) have been demonstrated previously.

Data on the backcrosses (table 2) show the same general result as in the F_2. In the backcross to *Langsdorffii* LLL is the only block which approaches the phenotype of the recurrent parent. In the backcross to *Sanderae* the combination SSS aproaches the phenotype of the recurrent parent.

In order to estimate the extent to which the segregating generations overlap the parental ranges only the tube base measurement was used. This single criterion is preferable for the purpose since it is least affected by environment and consequently the data more nearly represent the range of genetically controlled phenotypic expression. The results are shown in table 4. Less than one percent of the F_2 segregants overlap the parental range; and, since no correction was made for environmental fluctuation, it can be concluded that there is practically no recovery of parental types in the F_2. In the backcross to *Langsdorffii* 9 plants out of 116 or 7.8 percent overlap the range of *Langsdorffii*. In the backcross to *Sanderae* 14 plants out of 123 or 11.4 percent overlap the range of *Sanderae*. Summarizing, a small percentage of recurrent parent types for tube base are recovered in the first backcross generations, but neither parental type is recovered in the F_2.

RELATION BETWEEN POLLEN ABORTION AND MORPHOLOGICAL CHARACTERS

If pollen abortion is a factor in restricting recombination in segregating generations, through differential elimination of F_1 gametes, then it would be expected that the more extreme morphological recombinations recovered in the F_2 would show a higher percentage of pollen abortion than the more parental-like segregants. The mean percentage of pollen abortion, standard

*Standard error calculated as $\sqrt{\frac{pq}{n}}$.

error and range for each F_2 recombination block is shown in table 2. No consistent relation between blocks and pollen abortion was observed and appropriate calculations demonstrated that none of the differences between means is as great as twice the standard error. It was concluded that there was no evidence of a higher percentage of pollen abortion among male gametes of the more recombinative phenotypes than among the more parental-like combinations.

TABLE 3

Condensation of data in table 2 showing average percentage of pollen abortion in parental-like vs. all other classes of recombinations of corolla size characters. In addition, the percentage of pollen abortion is given for segregants with all parental color characters as well as parental-like size characters.

Generation	Most parental-like classes			All other classes	
	Type	Frequency	Pol. abor. (%)	Frequency	Pol. abor. (%)
F_2	LLL and SSS	116	32.7 ± 4.4	208	32.5 ± 3.2
	LLL and SSS with all P_1 or P_2 color char.	23	32.6 ± 9.8		
$F_1 \times$ *Langsdorffii*	LLL	28	41.2 ± 9.3	88	45.2 ± 5.3
	LLL with all P_1 color char.	5	29.0 ± 20.3		
$F_1 \times$ *Sanderae*	SSS	21	23.7 ± 9.3	102	36.7 ± 4.8
	SSS with all P_2 color char.	8	21.8 ± 14.6		

Further analysis of the F_2 data is shown in table 3. In addition to morphological distinctions the two parental species differ by a number of genes governing the inheritance of the flower and pollen color (SMITH 1937a). One or two major genes control each of these differences so that there is a total of at least nine color genes involved. Among the 116 F_2 segregants in the LLL and SSS groups 23 had all scorable color characters of one or the other parent species. These 23 individuals are the most parental-like of the 324 F_2's under study. The average percentage of pollen abortion in the 208 non-parental-like segregants is 32.5 ± 3.2 percent, in the 116 LLL and SSS blocks 32.7 ± 4.4 percent, and in the 23 segregants that were most parental-like in both corolla size and color 32.6 ± 9.8 percent. It is clear that within the range of F_2's recovered there was no correlation between pollen abortion and genes controlling phenotypic differences between the species. This sort of result is usually considered to show that the two types of hereditary characteristics are genetically independent (MÜNTZING 1930; STEBBINS 1950). However, the evidence is inconclusive for interpreting data on F_2's in which the parental types are not recovered. This point is discussed below.

Let us now consider the backcross results. From an analysis of the data

in table 2 for the $F_1 \times$ *Langsdorffii* it is evident that there is no significant difference in percentage of pollen abortion among the different blocks. The most parental-like block in this generation is LLL. In table 3 it is shown that the average percentage of pollen abortion for LLL segregants is 41.2 ± 9.3 and for all other blocks combined is 45.2 ± 5.3. Among the twenty-eight LLL plants five were classed as having all distinguishable color characters of *Langsdorffii*. The average percentage of pollen abortion for these five is 29.0 $\pm$ 20.3, which is markedly lower than in the other two groups but not statistically significant due to the small number of observations. This result at least suggests different interrelationships than were observed in the F_2.

TABLE 4

Frequency and extent to which segregating generations overlap parental classes for the character tube base.

Generation	Range (mm.)	Plants overlapping parental range	
		No.	Percent
Parental—largest *Langsdorffii* to smallest *Sanderae*	6–40		
F_2	6–36	2	0.6
$F_1 \times$ *Langsdorffii*	6–17	9	7.8
$F_1 \times$ *Sanderae*	20–47	14	11.4

In the backcross, $F_1 \times$ *Sanderae*, some significant differences in pollen abortion among blocks were found (table 2). For example, the difference between SSS (23.7 percent) and LLL (42.8 percent) is 24.5 ± 9.8. Segregants in block SSS are most like the recurrent parent and those in block LLL in this backcross most nearly resemble the F_1. Among the twenty-one SSS plants eight were indistinguishable in color characters from *Sanderae* (table 3). The average percentage of pollen abortion among these eight is 21.8 percent as compared to 23.7 percent in the SSS block as a whole and 36.7 percent in all other blocks combined. These differences, though not statistically significant, strongly suggest (as in the $F_1 \times$ *Langsdorffii*) that the most parental-like backcross segregants have a lower percentage of pollen abortion.

DISCUSSION

In segregating generations of the interspecific cross *Nicotiana Langsdorffii* $\times$ *N. Sanderae* both corolla size and pollen abortion show continuous variation and are considered to be governed in their inheritance by multifactorial genetic systems. In the F_2 generation the parental classes for corolla characteristics are not recovered and no correlation was observed between pollen sterility and different phenotypic recombinations. In the first backcross generations a small percentage of the segregants overlap the parental range of corolla characteristics. There was some evidence for significant differences in pollen sterility among the backcross recombination classes and the most parental-like segregants tended to have the lowest percentage of pollen abortion.

The genetic factors controlling different morphologies and pollen abortion could conceivably be the same, independent, or linked. Which of these interpretations best fits the data obtained?

In order to illustrate the type of result expected under each of the three conditions a simplified genetic model is shown in table 5. Two pairs of independent non-dominant genes with cumulative effects on corolla size are assumed and the size classes in the F_2 and a backcross are listed. If pollen abortion were controlled by genes independent of those for corolla size each

TABLE 5

Simplified hypothetical scheme showing the effect of three possible relationships between genes controlling morphological characters (A and B) and pollen abortion (C and D), namely: independence, pleiotropism, and linkage.

Gen.	Morphol. char.		Pollen abortion, percent*		
	Freq.	Genotypes	Independent	Pleiotropic	Linked A with C—20% c.o. B with D—10% c.o.
F_2	1	1 AABB	25	0	12.5
	4	2 AABb 2 AaBB	25	25	25
	6	4 AaBb 1 AAbb 1 aaBB	25	33.3	29.2
	4	2 Aabb 2 aaBb	25	25	25
	1	1 aabb	25	0	12.5
	Average		25	25	25
B_1	1	1 AaBb	25	50	42.5
	2	1 Aabb 1 aaBb	25	25	25
	1	1 aabb	25	0	7.5
	Average		25	25	25

*The genes for pollen abortion are assumed to be cumulative and to give 25 percent sterility when heterozygous and no sterility when homozygous at each locus.

F_2 size class would have statistically the same percentage of pollen abortion. If pleiotropic effects were involved there would be differences in percentage of pollen abortion among different F_2 size classes and segregants in the parental classes would be as fertile as the parents. If genes controlling corolla size were different from, but linked with, those causing pollen abortion then the situation in F_2 would be intermediate between that of independence and pleiotropism. If, instead of the simple genetic scheme outlined in table 5, many genes were involved and parental classes were not recovered in the F_2 then it would be difficult to distinguish among the three genetic systems postulated.

The experimental results on the F_2 reported in this paper do not provide evidence conclusive enough to clarify this point.

The backcrosses are more instructive. Under the hypothesis of independence (table 5) all corolla size classes would have statistically the same percentage of aborted pollen. Under the hypothesis of pleiotropism there would be marked differences among classes and the parental ones would be as fertile as the parent species. Under the hypothesis of linkage the percentages of pollen abortion would be different among corolla size classes (but less so than under pleiotropism), and the recovered parental classes would be least pollen sterile (but more so than the parents themselves). The experimental results obtained in these studies on the backcrosses (tables 2, 3 and 4) fit closest to the conditions expected with linkage. That is, there were significant differences in pollen abortion among a few of the backcross recombination types and the recovered parental-like segregants tended to have the lowest pollen sterilities which were, nevertheless, higher than in *N. Sanderae* or *N. Langsdorffii*.

The interpretation—*that the genetic factors causing partial isolation of the species though gamete sterility and those governing differences in morphological characters are individually discrete but linked*—is reasonable, for, if both systems are controlled by multiple factors located on only nine chromosomes, linkages would be inevitable.

With regard to differential restrictions on recombination of morphological characters in the interspecific F_2 the data presented indicate that gamete elimination, as measured by pollen abortion, is not an important contributing factor. The F_2 plants formed by combination of functional F_1 gametes constitute a rather wide range of morphological recombinations and the amount of abortion in their gametes shows no significant relation to relative degrees of hybridity indicated by the phenotype. Conclusive evidence of differential elimination would be difficult to obtain, however, since extreme recombinations are not recovered in the F_2 and hence their gametes cannot be studied. Linkages between factors affecting sterility and morphology would have relatively little effect on furthering differential restriction of morphological recombinations in the F_2 if, as the data indicate, highly heterozygous combinations are on an average no more sterile than those containing a considerable preponderance of genes from one or the other parental species.

Among backcross plants, formed by combination of functional F_1 female gametes with male parental gametes, no correlation of morphology with sterility was found, except in the most extreme parental-like combinations which contain genes of one parent almost exclusively. As a consequence of multifactorial inheritance a very small proportion of F_1 gametes would be as parental-like in genotype. It is concluded that gamete abortion is not an important factor in restricting recombination of morphological characters throughout a wide range of hybrid genotypes involving these two species of Nicotiana. The relatively greater fertility indicated in first backcross plants that are most like the recurrent parent species would be a factor in accelerating the rate at which subsequent backcrosses would approach the recurrent species.

SUMMARY

Interrelationships between morphological characters and pollen sterility were studied in the F_2 and first backcross generations of the interspecific cross *Nicotiana Langsdorffii* × *N. Sanderae*. The morphological characters used were three corolla dimensions that are determined by multiple factors. The combination of these characters in F_2 and backcrosses gives an estimate of the genetic composition of a phenotype with respect to relative amounts of parental germplasms. Pollen sterility is both chromosomal and genic; and many factors are involved in the inheritance as evidenced by the continuous variation in segregating generations.

F_2 segregation did not overlap the parental ranges in morphological characters and no correlation was observed between recombination types and pollen abortion. In the backcrosses some significant differences in pollen abortion among morphological recombination types were found and there was a tendency for segregants most like the recurrent parent to be least sterile. The results were interpreted to indicate that the genetic factors causing partial species isolation through gamete abortion are different from those governing the specific morphologies and that linkages occur between the two gene systems.

The data supported the interpretation that gamete elimination, as measured by pollen abortion, is relatively unimportant in differentially restricting morphological gene recombinations in F_1 gametes and F_2 segregants.

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THE F–V AND Z SYSTEMS OF BOVINE BLOOD GROUPS[1]

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EVIDENCE for two extensive series of alleles effecting blood groups in cattle has been given in a previous report (STORMONT, OWEN and IRWIN 1951). The two systems of blood groups are called B and C. Over 80 alleles are now recognized in the B series. The B alleles control so-called "groups" or "inherited combinations" of a minimum of 21 blood factors. For example, the B allele $B^{BO_1Y_2D'}$ controls the B group $B_{BO_1Y_2D'}$ comprised of a combination of four known blood factors B, O_1, Y_2 and D′ inherited "en masse" as a unit genetic character. Similarly, a minimum of 22 C alleles control the groups of inherited combinations of seven blood factors. Now, in the previous report (*loc. cit.*) 38 blood factors were utilized in the genetic analysis of the B and C systems, and, as pointed out, 21 of these were assigned to the B system and seven to the C system. Of the remaining 10 blood factors, all except the rare factor Z′ were excluded as members of both the B and C systems. The present report is concerned with the inheritance of three of these 10 remaining blood factors, F, V and Z. It is shown that two of these, F and V, are a pair of contrasting characters of a third system of bovine blood groups while the Z factor contrasts with its absence as a pair of groups in a fourth system.

MATERIALS AND METHODS

The development of antibodies in the iso-immunization of cattle for the bovine blood factors named F, V and Z has been described in previous reports (FERGUSON, STORMONT and IRWIN 1942; STORMONT 1950). Typing fluids or reagents for these three blood factors may also be prepared from rabbit antisera against cattle blood.

Hetero-immune sera produced in rabbits against the blood of individual cattle contain antibodies which react with the erythrocytes of all cattle and more specific fractions which react with the cells of some but not all of the individuals of the species. The latter fractions are separated from the former and from each other by absorption of the rabbit antisera with the erythrocytes of individual cattle or the pooled erythrocytes of several cattle. In addition to the fractions anti-F, V and Z, those for the bovine blood factors A, U

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(subtype U_1), S, E′ and H′ are more or less regularly encountered in rabbit antisera. More than half of the rabbit antisera in which antibodies for the factors F, V and Z were expected contained these antibodies in usable titers.

The antisera were prepared by injecting the rabbits intravenously with 0.5 ml of whole, citrated blood of individual cattle at intervals of two days for seven to nine injections. The serum was collected five to nine days after the last injection. The preserved antisera (merthiolate 1 : 2000) were kept at ordinary refrigerator temperature. Some of these antisera were used over a period of nine years as a source of typing fluids without any appreciable diminution in the reactivity of the fractions prepared from them.

For the absorptions, either trial absorptions or preparation of typing fluids, the antisera were usually diluted 1/8 or 1/16 and absorbed until the antibodies for the absorbing blood or bloods were exhausted. In general, this was accomplished in three successive absorptions of 10, 30 and 60–120 minutes duration at room temperature using a ratio of washed, packed erythrocytes to diluted serum of approximately 2 : 1. The final absorptions were chilled at refrigerator temperatures prior to centrifugation and separation of the absorbed serum. The reagents were stored in a freezer. Reagents prepared only from rabbit antisera were used throughout the present study.

The bovine blood-typing test is essentially a hemolytic test. Cattle erythrocytes sensitized with antibodies of the typing fluids exhibit no visible manifestation of the reaction unless complement is present. Rabbit complement has been used throughout all of these studies. The method of performing the tests has been given in the papers already cited (also see FERGUSON 1941; STORMONT and CUMLEY 1943).

EXPERIMENTAL

The F–V system of bovine blood groups

While the anti-F reagents from rabbit antisera reacted with the same bloods as the original F reagent from iso-immune antiserum (STORMONT 1950), these reactions differed from those obtained with the latter reagent in that two kinds of reactive bloods could be readily distinguished by their rates or degrees of reaction per given interval of time. Those bloods referred to as "strongly reactive" were rapidly and completely or nearly completely hemolysed soon after the set-up of the tests, while those bloods referred to as "weakly reactive" were only slowly and less completely hemolysed within the ordinary time limits of the tests (final readings usually three and one-half to four hours after the set-up). Differences only in the degree of manifestation of these strong and weak reactions were noted in a comparison of seven different F reagents prepared from as many different rabbit antisera. In general, the differentiation of the strongly and weakly reactive bloods was the sharpest with the more potent reagents (titers greater than 1/256) and in the lower dilutions (usually 1/32 and 1/64) of all the reagents. The most accurate distinction of the two kinds of reactive bloods was made in the early readings of the tests, usually 10 to 30 minutes after the onset of hemolysis. Readings

taken at 45 minutes to one hour after the onset of hemolysis, or about the time the cells had settled, occasionally included some of the weakly reactive bloods among those considered strongly reactive, or the converse.

Attempts to improve this distinction of the two kinds of F-positive bloods by partial absorption of rabbit F reagents with weakly reactive cells were not successful. On the contrary, both kinds of bloods readily absorbed the F antibodies. Quantitative absorptions demonstrated that the strongly reactive bloods were more efficient than the weakly reactive bloods in absorbing a given amount of F antibodies. These results suggested that the erythrocytes of strongly reactive bloods contain more of the antigen than those of the weakly reactive bloods, which might be a consequence of homozygosity of the gene controlling the F factor.

The possibility that the strongly and weakly reactive F-positive bloods could be respectively from individuals homozygous and heterozygous for a single gene (F) was first tested by means of gene-frequency analyses. The data were drawn largely from a study of the bloods of Guernsey and Holstein-Friesian cattle sent to this laboratory for blood-typing tests in connection with

TABLE 1

A gene-frequency analysis of the distribution of strongly and weakly reactive F-positive bloods in two breeds of dairy cattle.

Breed and number of individuals sampled		Reactions			F Gene-frequency	Chi-square
		F-strong	F-weak	F-negative		
Holstein-Friesian	1488	1115	339	34	87.0	2.54
Guernsey	2024	1768	245	11	93.4	0.66

problems of parentage arising in registration. While some randomization was assured, no attempt was made to weight the data on the basis of family relationship of the animals. While the present data (table 1) do not carry the same precision as those based on unrelated individuals (COTTERMAN 1947), they are considered adequate to test the hypothesis.

A summary of the data on bloods of 1488 Holstein-Friesians and 2024 Guernseys is given in table 1. For each breed, estimates of the frequencies p and q of genes F and f are first derived as $p = (2a + b)/2n$ and $q = (b + 2c)/2n$ where a, b and c are the observed numbers of the animals presumed to correspond to the genotypes FF, Ff and ff, and where the total is denoted by $n = a + b + c$. These estimates, which amount to actual counts of the two genes detected in each individual are maximum likelihood estimates. Using them, one may compare the observed frequencies, a, b, c, with the numbers expected under simple genetic equilibrium, viz., np^2, $2npq$ and nq^2, respectively, using the standard formula for chi-square. However, a simpler direct formula, obviating the need for computing expected numbers, has been given by WIENER (1935). A single degree of freedom remains for testing the genetic theory, since, although there are three observed classes, two degrees

of freedom are lost, one for making the expected total equal to the observed n, the other for adjusting the expected and observed series so as to agree in the proportion of F genes, p.

As is seen from table 1, the data for each breed are in satisfactory agreement with the two-allele theory, the value of chi-square being in each case less than the chi-square value at the $P = 0.05$ level. The difference in gene frequency between the two breeds is significant.

Tests had been completed on 1425 bloods over a period of more than a year before the observation was made that bloods which were positive with V antibodies were either weakly reactive or negative with F antibodies, while V-negative bloods were strongly reactive with F antibodies. This suggested that the gene for V is the allele of that for F. Perusal of the results of the classification of the 1425 bloods that had been tested prior to noticing this relationship provided an unbiased test of the accuracy of these classifications on the assumption that the gene for V is the allele of that for F. Only ten

TABLE 2

The inheritance of the bovine blood factors F and V as alternatives.

Kind of matings (genotypes of parents)	Number of progeny of blood types		
	F (genotype FF)	FV (genotype FF^V)	V (genotype F^VF^V)
$FF \times FF$	669	0	0
$FF \times FF^V$	113	119	0
$FF \times F^VF^V$	0	22	0
$FF^V \times FF^V$	21	36	21
$FF^V \times F^VF^V$	0	16	15
$F^VF^V \times F^VF^V$	0	0	0

possible exceptions were noted. Several of the V-positive bloods had been classified as strong F, and several of the V-negative bloods had been classified as weak F. None of the bloods lacked both of these factors. Since these earlier tests, no established exceptions to the rule were noted. The overall results indicate that the explanation given above is correct and that the earlier exceptions were errors of classification. It should be pointed out that in the diagnosis of strongly and weakly F-positive bloods, twins, triplets, etc., possessing an admixture of blood cells (OWEN 1945; OWEN, DAVIS and MORGAN 1946) present a special problem which will not be discussed in this report.

Additional evidence supporting this explanation has come from a study of the inheritance of F and V blood groups in 1032 offspring. The data are summarized in table 2. The gene-symbols F for the F allele and F^V for the V allele are used. The kinds of offspring and ratios resulting from the various kinds of matings are in agreement with the theory. There were no matings in which both parents were homozygous for the F^V allele.

The Z system of bovine blood groups

As given in the foregoing example of the differences in reactivity of F-positive bloods, Z reagents prepared from rabbit antisera were used to classify Z-positive bloods into two kinds, "strongly reactive" and "weakly reactive." The quantitative aspects of the reactions paralleled those already mentioned for the F factor. A gene-frequency analysis of the results of tests on the bloods of 1482 Holstein-Friesians and 2047 Guernseys is given in table 3. These results support the proposal that the strongly and weakly reactive Z-positive bloods are, respectively, from individuals homozygous and heterozygous for a single gene, hereinafter referred to as the *Z* gene or allele. The frequency of this gene differs significantly in the two breeds.

TABLE 3

A gene-frequency analysis of the distribution of strongly and weakly reactive Z-positive bloods in two breeds of dairy cattle.

Breed and number of individuals sampled		Reactions			Z Gene-frequency	Chi-square
		Z-strong	Z-weak	Z-negative		
Holstein-Friesian	1482	163	630	689	32.0	1.15
Guernsey	2047	542	1043	462	52.0	0.87

Antibodies which react specifically with the product(s) of the allele(s) of *Z* have not been developed. Taking into account the number of antisera studied in which these hypothetical antibodies could have been expected, it might be assumed, if the alternative of Z is antigenic, that it only engenders antibodies which are completely cross reactive with Z. It will be convenient, however, to assign the symbol *z* for a proposed allele whose product has not or cannot evoke antibodies with which it alone will react.

Data on the inheritance of the Z blood groups in 1032 offspring are given in table 4. The genotypes shown in table 4 are those classified directly from

TABLE 4

*The inheritance of strongly and weakly reactive Z-positive bloods of cattle.**

Kind of matings		Number of progeny of blood types		
Phenotypes	Genotypes	Strong Z (ZZ)	Weak Z (Zz)	Z-negative (zz)
Strong Z × Strong Z	ZZ × ZZ	17	0	0
Strong Z × Weak Z	ZZ × Zz	63	59	0
Strong Z × Z-negative	ZZ × zz	0	71	0
Weak Z × Weak Z	Zz × Zz	50	118	49
Weak Z × Z-negative	Zz × zz	1 (?)**	194	189
Z-negative × Z-negative	zz × zz	0	1 (?)**	220

* The Z genotypes were inferred directly from the results of the serological tests with differential Z reagents prepared from rabbit antisera.

** See text for possible explanations of these apparent exceptions.

the results of the serological tests with Z reagents prepared from rabbit antisera. Only two exceptions are noted (table 4) in which the proposed genotypes of the 1032 offspring were not in agreement with the kinds expected on the assumption that the proposed genotypes of the putative parents were always correct. In both instances check tests confirmed the original classifications. The one offspring which possessed the Z factor from putative parents, both of which lacked that factor, might be considered as illegitimate. The other exception, an individual whose cells were strongly reactive (genotype *ZZ*) from a proposed mating of $Zz \times zz$, could be considered a possible exceptional individual of genotype *Zz* whose cells were, nevertheless, strongly reactive. The possibility of illegitimacy could not be ruled out in either exception. Neither, of course, could mutation be excluded.

DISCUSSION

The studies of systems of blood groups, examples of which in species other than cattle, are M–N and Rh of man (LANDSTEINER and LEVINE 1928; LANDSTEINER and WIENER 1940; WIENER, SONN-GORDON and HANDMAN 1947), and A and B of chickens (BRILES, IRWIN and MCGIBBON 1950), have invariably shown that the blood groups of a system segregate as the products of allelic genes. The number of alleles may range from two as in the initial proposal of the M–N system of man, or the F–V and Z systems of cattle, to upwards of 80 as in the B system of cattle (*loc. cit.*). Without an established exception there appears to be a one-to-one correspondence between blood group antigens and their controlling genes, and this irrespective of the numbers of blood factors that may characterize the individual antigens. The observations of IRWIN (1932), IRWIN and CUMLEY (1945), MCGIBBON (1944) which imply gene-interaction in the production of at least some of the antigenic properties of the erythrocytes of certain interspecific hybrids in birds have as yet had no parallel within the known blood groups of individual species. Likewise, FISHER's proposal (1947) that the Rh system of man involves three closely or absolutely linked loci does not disagree with the one gene–one antigen correspondence, since a corresponding sub-division of this system into three elementary systems called C, D and E was also proposed.

Perhaps the main problem involved in the classifications of systems of blood groups has been that of interpreting the serological data, namely, the relative specificities of the antibodies (blood-typing reagents) used in the studies. This problem has considerable bearing on the genetic interpretation of those systems embracing more than two blood factors. Serological typing alone, of course, involves the interpretation of one unknown, the antigenic structure of cells, in terms of another, antibodies whose cross-reactive potentials are likewise unknown.

It is perhaps significant in connection with this problem that in none of the more complex systems of blood groups (B and C of cattle, A of chickens and Rh of man) has it been possible to develop antibodies specific for the individual antigens to the mutual exclusion of all others of a system. These

results would appear to fortify the modern concept of antibody, namely, " not to serum globulin modified in any single manner, but to a series of modified globulins separable and identifiable as distinct fractions and limited in number mainly by their cross reactivities and the quantitative criteria available for their characterization " (from HEIDELBERGER, KABAT and MAYER 1942; also cf. LANDSTEINER 1936, 1945). The conclusion may be drawn that the blood-group antigens of a system are, generally, a series of two or more chemically, closely related substances with greater or lesser degrees of overlapping specificities.

Most of those systems of blood groups which embrace only a single pair of alleles do not offer the difficulties of serological interpretation inherent in the more complex systems. Confining the studies to the erythrocytes of the one species being investigated, there are two general serological possibilities. (1) If the members of the pair of antigens are sufficiently distinct from each other as antigens, it will be possible to develop antibodies which will clearly differentiate the two. All the reagents which react with one of the allelic antigens but not the other need not contain the same kinds of antibodies or be regarded as mono-specific fractions or populations of antibodies. That such reagents will appear to be the same in parallel tests, or to behave as rigidly specific antibodies in absorptions, is a consequence of the limiting genetic situation, that is, only one antigen, the homologous antigen, is involved in the reactions. (2) If one of the pair of antigens is not capable of engendering antibodies or only gives rise to antibodies that are completely cross reactive with its alternative it will not, of course, be possible to develop reagents specific for this antigen to the exclusion of the other. The presence of the incompletely differentiated antigen may then be recognized by its failure to react with those reagents specific for its alternative or, in certain systems, by a clear-cut reduction in the rate and degree of reaction of its alternative in the cells of heterozygotes. The F–V system of this report conforms, now, to the first of the two serological possibilities set forth while the Z system would appear to be an example of the second.

Differences in the sensitivity of the blood cells of different individuals when treated with the same antibodies have been noted in the studies of blood groups of other species and some of these differences, as the two examples involving the blood factors F and Z of this report, are known to be a consequence of differences in homozygosity or heterozygosity of the individuals for the particular genes concerned. For example, LANDSTEINER and LEVINE (1928) noted that human bloods from individuals homozygous for the N agglutinogen were more strongly agglutinated, as a rule, than were the bloods of heterozygotes. A similar but less pronounced difference was noted for the alternative M. The observation regarding the M agglutinogen, while bearing the same genetic relationship to N in man as V bears to F in cattle, stands somewhat in contrast with those concerning the sensitivity of the V antigen of cattle. Repeated tests with a number of anti-V reagents prepared from both iso and hetero-immune antisera have not shown any appreciable differ-

ences in the reactions of the cells of cattle homozygous and heterozygous for the V allele. Additional examples in man are to be found in the various papers (not all cited here) dealing with the Rh (CDE) groups of man. For example, hr′ reagents (also called anti-c) produce stronger reactions with the cells of those individuals in which alleles for the blood factor hr′ (or c) are present in double dose (RACE, TAYLOR, BOORMAN and DODD 1943). OWEN (1948) reported the existence of a pair of contrasting cellular characteristics in rats in which the heterozygote behaved as if it were intermediate in number of respective antigenic sites per cell. OLSON (1943) noted in chickens that the erythrocytes of individuals homozygous and heterozygous for apparently a single agglutinogen (blood factor) may be differentiated serologically.

The usual explanation for these differences in sensitivity of the blood cells has been that two doses of the gene produce more of the same antigen than does one dose. While the effects may be purely quantitative in all of these examples, the explanation based on dosage-effects alone may not be invariably correct. The phenomenon can also be understood on the principle of competition of alleles or their products for the same substrates. OWEN (1948) stated that the pair of alleles in rats may compete for a common substrate. In the two examples given in this report, the reduced effectiveness of the genes *F* and *Z* has been associated with the presence of their respective alternatives F^V and *z*. The possibility of allele-competition cannot be ruled out and may eventually be put to test if, for example, individuals with chromosome-complements of 2n–1 should become available for comparison.

Most of the F and Z reagents developed to date from rabbit antisera may be used singly (*i.e.*, not in conjunction) to distinguish the cells of homozygotes and heterozygotes for the respective genes *F* and *Z* but it has not often been possible to do this using F and Z reagents prepared from iso-immune antisera. Until recently, however, only one F reagent from an iso-immune serum was available for study. It is possible, if differences do exist in this respect, that the explanation may involve both qualitative and quantitative differences in the kinds of F and Z antibodies produced by the two species (rabbits and cattle). KABAT (1939) demonstrated that anti-pneumococcus sera produced in cattle and rabbits differed greatly in respect to the molecular weights of the antibodies. The antibodies (molecular weight of 990,000) produced in cattle were about six times heavier than those produced in rabbits. Assuming that smaller antibodies might be bound in greater multiples per unit of antigen, it is possible that the different rates of hemolysis noted in the present study could be accentuated. That other unknowns may be involved is indicated by some recent observations made with the aid of another iso-immune antiserum containing F antibodies. This antiserum, like the original iso-immune F reagent, produced about the same rate and degree of hemolysis with all F-positive bloods. However, when this new F reagent was heated at 56°C in a water bath for approximately 24 hours, it no longer produced hemolysis (or only a negligible trace) of the cells of heterozygotes (FF^V) in the ordinary time-limits of the tests. On the other hand, the heated antiserum

was only slightly less effective in its hemolytic action on the cells of homozygotes (FF). The heat-treated antibodies were readily bound by the cells of heterozygotes as demonstrated by absorption tests. The differences in the reactions obtained with the heat-treated as compared with the unheated antiserum are probably primarily the result of partial denaturation of antibody-globulins by the prolonged heating; the secondary manifestations of the reactions, associated with the genotypes FF^V and FF^V, are not understood.

SUMMARY

1. Evidence for the third and fourth systems of bovine blood groups is presented. Each of these systems, the one named F–V and the other Z, involves at the present only a single pair of alleles. The pair of alleles designated F and F^V control the three phenotypes F, FV and V of the F–V system. Only one of the two proposed characters of the Z system, *i.e.*, the antigen designated Z, is known to evoke antibodies that will react with it but not its alternative. The gene-symbol z is proposed for the allele whose product does not or has not given rise to antibodies that will react with it alone.

2. By the use of F and Z reagents (antibodies) from certain rabbit antisera it is possible to differentiate the bloods of individuals homozygous for these two genes, respectively. This direct serological criterion for homozygosity as distinguished from heterozygosity is based on sharply defined differences in rates of hemolysis. For example, the bloods of each of the homozygotes FF and ZZ are rapidly and completely hemolysed while those of each of the heterozygotes are only slowly and often less completely hemolysed within the time-limits of the tests. All three genotypes in each of these two systems can be classified by the use of only one reagent for each system. The differences in rates of hemolysis, believed to have a purely quantitative basis in these two examples, are discussed.

3. Differences in the frequency of the alleles of these two systems are noted in two breeds of dairy cattle.

4. These results have immediate practical application in connection with problems involving parentage in cattle.

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PENETRANCE AND EXPRESSIVITY OF TUMOROUS HEAD IN DROSOPHILA MELANOGASTER AND RELATIVE VIABILITY OF FLIES CARRYING TUMOROUS HEAD GENES

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AN hereditary head abnormality in *Drosophila melanogaster* and the genetic mechanism which controls it were discussed by GARDNER and WOOLF (1949) and GARDNER (1948b, 1949). The influence of temperature treatments on the expression of the character was described by GARDNER and WOOLF (1950) and WOOLF (1949). The objective of the present paper is to discuss the penetrance and expressivity of the character and the relative viability of the flies carrying the genes upon which the expression of the trait is dependent.

MATERIALS AND METHODS

The material used for this study was a stock of flies (*Drosophila melanogaster*) carrying an hereditary abnormality characterized by growths in the head region. The history of the stock and description of the phenotype were given by GARDNER (1948) and NEWBY (1949). The term tumorous head (symbolized *tu-h*) has been used as a descriptive name for the stock. The inheritance of the abnormality is dependent upon two genes. One (*tu-1*) is sex-linked producing a maternal effect and the other (*tu-3*) is semidominant and is located in the third chromosome. The inbred tu-h stock is homozygous for both genes. The penetrance in the inbred stock is about 76 percent at 22°C. Outcrosses involving females from the tu-h stock and males from laboratory stocks produced an average of about 30 percent of abnormal flies in the F_1 at 22°C. Reciprocal outcrosses between tu-h males and females from laboratory stocks, other than Oregon R, Stephenville and Crimea, produced less than one percent of abnormal F_1 flies. The three wild stocks (Oregon, Stephenville and Crimea) which did not conform to the usual pattern were shown by GARDNER and WOOLF (1949) and GARDNER and STOTT (1951) to carry genes influencing the expression of the trait. Small percentages, not exceeding five percent of abnormal flies, were obtained in the F_2 from both reciprocal crosses except in the presence of modifiers (DEARDEN, 1949; GARDNER and STOTT 1951).

The eggs for the viability studies were collected on agar blocks 1″ by 3″ by ⅛″ containing sugar and covered by a thick yeast suspension. The blocks were placed on moistened blotter pads covering microscope slides of the same size. The entire unit was introduced into a one-half pint milk bottle in which 400–500 flies were kept. In later experiments, females five to seven days old

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were selected and only 30–40 were necessary to produce sufficient eggs. The bottle was then placed in the incubator in a horizontal position. At regular intervals eggs were transferred individually, on the point of a needle, to fresh, moist blotter pads ⅛″ square, which were covered with thick yeast. Twenty-five eggs were placed on a pad. Each pad was then inserted into a 1″ by 4″ shell vial and placed on the slanting surface of the food medium previously introduced into the vial. The standard cornmeal agar molasses medium was used for all experiments. A constant temperature was maintained and all other environmental factors were controlled as well as possible. The larvae crawled off the blotters into the food medium soon after they hatched. The blotter pad and food medium were kept moist. The unhatched eggs were counted to determine the percentage of eggs hatched. The adults were removed and classified for the tumorous head character as soon as they emerged in the vials.

EXPERIMENTAL RESULTS

Penetrance

So far it has been impossible to secure a pure breeding strain with 100 percent penetrance (all flies showing the external abnormalities). From a recent series of crosses involving forty-four single pair matings from the inbred stock at 25°C, an average of 79 percent of the flies showed external expressions. The range was from 51 percent to 94 percent and a fairly normal distribution was indicated. Internal abnormalities not visible on the surface have also been observed (NEWBY 1949). An increase of about 15 percent in penetrance has been obtained by selection over a period of four years. Comparable large scale studies made at successive intervals of about one year at 25°C have shown an average penetrance of 64, 74 and 79 percent. It is presumed that modifiers have been accumulated to account for this increased penetrance. Evidence presented by GARDNER and WOOLF (1949) indicated that the second chromosome of the tu-h stock carried a modifier or modifiers increasing the penetrance by about ten percent. Modifiers for the tumorous head character have also been found in several stocks (DEARDEN, 1949; GARDNER and STOTT 1951).

The Stephenville stock was shown by GARDNER and STOTT (1951) to carry a sex linked gene (*tu-1*^{s}), presumably an allele of *tu-1,* which exerted a more powerful maternal effect than *tu-1.* A new stock was synthesized in which *tu-1* was replaced by *tu-1*^{s}. A penetrance of 90 percent was obtained at 22°C. The new stock was inbred for twelve generations without any appreciable change in penetrance.

The penetrance of the visible trait was observed to be significantly greater among the females than among the males in the inbred tu-h stock at all temperatures studied between 15°C and 31°C. From one series of 24 single pair matings maintained at 25°C a total of 1914 flies was produced. The penetrance of the visible expression ranged from 60 percent to 90 percent in the progeny resulting from individual crosses. The average penetrance for the total was 80 percent. The average penetrance among the females was 88

percent, compared with 74 percent for the males. This is a consistent difference observed repeatedly in further studies. No significant difference was observed in the results of crosses between abnormal parents and those between normal appearing parents from the inbred tu-h stock.

Environmental factors as well as genetic modifiers are known to influence the penetrance. GARDNER and WOOLF (1950) have shown a regular increase proportional with temperature from 54 percent of abnormal flies at 18°C to 93 percent at 30°C during an early temperature effective period. Results of reciprocal crosses showed that it was the maternal effect which was influenced by temperatures. Other environmental factors, such as quantity and quality of the food, have also been observed to influence penetrance. When stock bottles became old the incidence of head growths was decreased and the individual growths observed were smaller and less conspicuous. It has also been observed that crowding decreases penetrance. This is probably the result of selection against the weaker tumorous head phenotype.

Expressivity

Wide variation was observed in degree of abnormality in individual flies expressing the trait (Plate 1, figures 1–9). Some irregularities were very small involving only a few facets of the eye or a small area elsewhere on the head. All gradations in size were observed from small growths or pits to massive abnormalities occupying most of the head. One expression was a crater-like pit in the eye, often with a growth in the bottom. Usually, however, there was an irregular enlargement or evidence of tissue destruction by an abnormal growth. Most tumors were amorphous but some were leg-like, suggesting homoeosis. A few elaborate leg-like structures have been observed with as many as three distinct segments and claws extended distally.

The growths were not localized in any one region of the head, but were distributed quite uniformly in the areas normally occupied by the eye, face, antenna and base of the mouth parts. An illustrated description of the different expressions of the character has been prepared by NEWBY (1949). Some variations of the phenotype bear a resemblance to well known mutants such as: ophthalmopedia (BRIDGES and BREHME 1944), Eyeless-dominant (PATTERSON and MULLER 1930), erupt (GLASS 1944), Deformed (BRIDGES and MORGAN 1923) and aristapedia (BALKASHINA 1929). The main differences between tumorous head and these mutants were in the distribution of the abnormalities on the surface of the head and the wide variation in size and shape. The tumorous head growths were not localized in any one region and a wide variety of expressions was observed.

The expressivity in the two sexes was studied by classifying 1914 flies from pair matings between tumorous head flies on the basis of the degree of visible abnormality. The normal flies were first separated from the abnormals. The flies showing tumors were then classified into five classes according to the proportion of the surface of the head which was distorted by the growths. Class 1 included flies with only one small growth; class 2, flies with two

PLATE 1

Expressions of Tumorous Head

FIGURE 1.—Pit in center of right eye with abnormal growth at the bottom.

FIGURE 2.—Abnormal growth projecting upward from anterior dorsal part of left eye.

FIGURE 3.—Abnormal growth replacing lower part of left eye and extending ventrally below eye.

FIGURE 4.—Two abnormal growths replacing lower margin of right eye and extending ventrally.

small growths or one larger one not exceeding one-half the size of the normal eye; class 3, flies with three small growths or a total distorted area large enough to cover the area occupied by one-half to one whole normal eye; class 4, flies with approximately one-half the surface of the head distorted and class 5, flies showing massive defects involving most of the surface of the head. In addition to the increased penetrance among females from the inbred stock, a higher degree of abnormality was observed among the individual females than among the males. The curves shown in figure 10 summarize the expres-

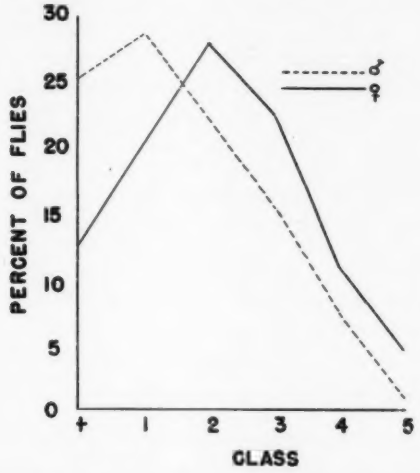

FIGURE 10.—Expressivity in male and female flies from the inbred tu-h stock. Class 1, one small growth; class 2, two small growths or one larger one not exceeding one-half size of normal eye; class 3, three small growths or total distorted area large enough to cover one-half to whole normal eye; class 4, approximately one-half of surface of head distorted; class 5, massive defects involving most of the surface of the head.

sivity for the two sexes. The data show a higher percentage of normals among the males and a higher degree of expression among the females. Extreme abnormalities were more frequent among the females than among the males. Temperature treatment and other environmental factors also were shown to influence expressivity. Relatively lower numbers of flies and abnormal sex ratios associated with higher expressivity at increased temperature suggested

FIGURE 5.—Abnormal growth replacing posterior, ventral quarter of left eye.

FIGURE 6.—Abnormal growth replacing anterior medial section of left eye and extending medially.

FIGURE 7.—Abnormal growth replacing large portion of left eye and other head parts.

FIGURE 8.—Ventral view of abnormal growth extending anteriorally from medial margin of right eye above the base of the mouth parts.

FIGURE 9.—Leg-like growth extending anteriorally from base of left antenna.

The photographs were prepared by L. Jay Smith.

that increased expressivity produced lethals. When observing the abnormal flies, it was not difficult to visualize how a slightly greater abnormality than those observed in some living females would make the flies unable to survive.

Viability

The numbers of progeny produced from matings between tu-h flies were observed to be lower than those from other laboratory stocks kept in the same environment. A significant difference in the sex ratio was also observed at 25°C. This differential became more pronounced at temperatures above 25°C. From a total of 1914 tu-h flies raised at 25°C, 858 were females and 1056 were males. This represents a highly significant deviation from the expected sex ratio, observed among the controls from normal stocks. At higher temperatures the sex ratio was more markedly abnormal. This suggested a differential viability associated with sex. The experiments described below were designed to study the relative viability of tumorous head flies more critically.

The first experiment was designed to compare the viability of flies from the tu-h, Oregon R, and Lausanne S stocks. Preliminary results were reported by RATTY (1949). Eggs were collected as described above and transferred in groups of 25 to blotter pads covered with yeast. The percentage of larvae and adults from a given number of eggs was determined and the adult flies were classified for the tumorous head abnormality. The temperature was maintained at 21°C.

TABLE 1

The percentage of larvae and adults obtained from tu-h, Oregon R, and Lausanne S stocks.

Stock	Eggs collected	% eggs hatched	Adults	% eggs to reach adult stage	% larvae to reach adult stage
			21 ± .5° C		
tu-h	4400	70	2210	50	72
Oregon R	3425	91	2543	74	82
Lausanne S	2800	86	1992	71	83
			30 ± .5° C during early developmental period		
tu-h	4000	35	232	6	17
Oregon R	3475	63	1801	52	83
Lausanne S	3250	66	1788	55	83

Table 1 summarizes the results from this experiment. An average of 70 percent of the tu-h eggs at 21°C hatched. The range for individual groups of eggs extended from 48 to 83 percent. The average for Oregon was 91 percent with a range from 82 to 97 percent, and that for Lausanne was 86 percent with a range from 80 to 94 percent. These results showed that the average number of tu-h eggs to develop into larvae was about 20 percent lower than that of either Oregon or Lausanne. The early developmental period at which this loss occurred was the period at which the maternal effect was expected to occur. This period also coincided with the temperature effective period

described by GARDNER and WOOLF (1950). Fairly comparable results were obtained from Oregon and Lausanne. Oregon was shown in a previous study to carry the *tu-1* gene. This result indicated that *tu-1*, when alone, has no effect on the viability of the flies which carry it.

Unfortunately the tu-h stock was not isogenic when these studies were made. It is possible that other genetic factors carried in the stock were responsible for the differences observed. Since the data cited below from the results of outcrosses indicated a maternal effect in viability as well as penetrance it was assumed until further evidence is available that the difference was determined directly by the tu-h genes or through an interaction in which these genes were involved.

A comparison of the percentages of adults obtained at 21°C showed an additional loss of about 20 percent for all three stocks. An average of 50 percent of the tu-h eggs reached the adult stage. The range for individual groups was 28 to 64 percent. The average for Oregon was 74 percent with a range of 63 to 82 percent and that of Lausanne was 71 percent with a range of 62 to 83 percent. When the percentages of newly hatched larvae which reached the adult stage were compared, an additional relative loss was observed for tumorous head. About 72 percent of the tu-h larvae reached the adult stage compared with 82 percent for Oregon and 83 percent for Lausanne. The relative loss in the egg stage of tu-h was about twice as great as that during the rest of the entire life cycle from early larva to adulthood. Again, at this later stage, Oregon was comparable with Lausanne, indicating that the *tu-1* gene alone does not lower the viability of the flies which carry it at the later developmental period. This suggested that the adverse effect on viability characteristic of the tu-h stock was caused by the *tu-3* gene or an interaction involving *tu-1* or *tu-3*, or their products. The tu-h adults were classified for the tumorous head abnormality as a check to compare with previous experiments. An average of 65 percent of abnormal flies was obtained. This was within the expected range at 21°C. The proportion of abnormal males and females also followed the expected pattern.

Since a temperature treatment at 30°C during an early temperature effective period had been shown to increase the penetrance of the tumorous head expression, an experiment was next designed to determine whether or not the viability was changed by treatment at 30°C during this period. The experiment was conducted like the one at 21°C but the eggs were laid at 30°C and replaced in the 30°C incubator for the period immediately following. The total period of temperature treatment was 19 hours. Because of technical difficulties, some eggs did not receive the treatment for the entire period. All eggs were exposed to 30°C for at least 3 hours. The experimental cultures and controls received the same treatment in all cases. The results are summarized in table 1. An average of 35 percent of the tu-h eggs hatched. The range extended from 24 to 45 percent. The average for Oregon was 63 percent, with a range from 21 to 84 percent, and that for Lausanne was 66 percent, with a range from 27 to 84 percent. Again the average percentage of

tu-h eggs to hatch was considerably lower than that of the other two stocks. Oregon and Lausanne were fairly similar, indicating that *tu-1* alone had no effect on viability under this treatment. The average penetrance for the treated flies was 73 percent. This is considerably lower than that (93 percent) obtained by GARDNER and WOOLF (1950) when all eggs were treated at high temperature for the entire 24 hours.

An average of 6 percent of the tu-h eggs reached the adult stage when 30° temperature treatment was applied. The range was from .5 to 21 percent. The average for Oregon was 52 percent, with a range from 14 to 76 percent, and the average for Lausanne was 55 percent with a range from 12 to 72 percent. Considerably wider variation was observed among individual groups representing all three stocks under the influence of temperature treatment, than at normal temperature. The average viability for the tu-h stock was considerably lower than that for Oregon and Lausanne. Only 17 percent of the tu-h larvae became adults, compared with 83 and 83 percent, respectively, for Oregon and Lausanne. A considerably lower relative viability was thus demonstrated for tu-h in the later developmental periods, following heat treatment, as well as in the egg stage, during which the heat was actually applied. This shows that the influence of the early heat treatment on viability extends into later developmental periods. These results are shown graphically in figure 11.

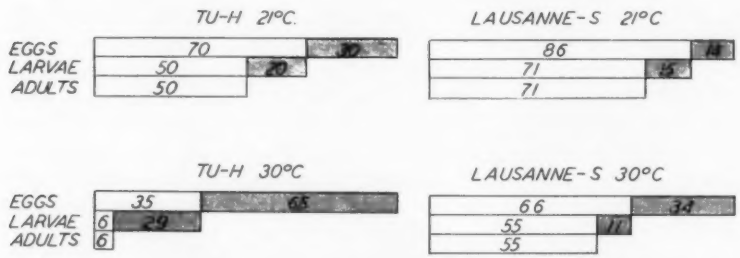

FIGURE 11.—Relative viability of tu-h and Lausanne S.

A marked differential sex viability was also observed in the tu-h stock following heat treatment. The data show that only 232 of the 4000 tu-h eggs reached the adult stage. Of these adults, 52 were females and 180 were males. From a comparable experiment involving treatment at a temperature (31°C) one degree higher, GARDNER and WOOLF (1950) obtained only 17 females and 232 males. Since greater penetrance and expressivity were observed among the females than among the males at temperatures above normal and increased temperature at the early developmental period was shown to increase the penetrance and expressivity and decrease viability, it seemed likely that a greater proportion of abnormal flies (mostly females) did not survive the treatment.

A maternal effect had been demonstrated with reference to the incidence of the tumorous head abnormality from the results of reciprocal outcrosses

(GARDNER and WOOLF 1949). The next experiment was designed to determine whether or not a comparable effect on viability could be detected. Reciprocal crosses between tu-h and Lausanne were prepared for this experiment. The results (table 2) show a significant difference in viability of flies resulting from reciprocal crosses. From the cross between tu-h females and Lausanne males 2800 eggs were collected, of which 78 percent hatched into larvae. From the reciprocal cross between Lausanne females and tu-h males, 2650 eggs were collected and 95 percent hatched. These results indicated a difference in viability as well as penetrance from reciprocal crosses. The differential viability may also be attributed to a maternal effect. A difference in viability of the larvae was reflected in the percentages of adults produced from the reciprocal crosses, but no important decrease in relative viability was observed between the larval and adult stages. This shows that the major processes influencing viability occur at the same early period of development as those reflected in the expression of the tumorous head abnormality.

TABLE 2

Percentage of larvae and adults obtained in F_1 and F_2 from reciprocal crosses between tu-h and Lausanne S.

	Cross	Eggs collected	% eggs hatched	Adults	% eggs to reach adult stage
		21 ± .5° C			
F_1	*tu-h × Lausanne	2800	78	1889	67
	Lausanne × tu-h	2650	95	2223	84
		23 ± .5° C			
F_2	tu-h × Lausanne	1000	70	664	66
	Lausanne × tu-h	1425	76	882	62

* Female parent is always written first.

As shown in table 2, smaller differences were observed in the F_2 from the original reciprocal crosses. From a total of 1000 eggs collected from the F_1 of the cross between tu-h females and Lausanne males, 70 percent hatched and 66 percent reached the adult stage. From the 1425 eggs collected from the F_1 of the reciprocal cross, 76 percent hatched and 62 percent became adults. When these results were examined for independence, chi-square values of 9.55 and 5.36 were obtained for the eggs hatched and eggs which became adults, respectively. These values are both significant at the .05 point. Since the percentages vary in opposite directions and since the chi-square values were within the range obtained from individual cultures and groups of cultures within each total, the variation between reciprocal crosses in the F_2 was attributed to minor environmental variation. For comparison, the chi-square values obtained when the F_1 reciprocal crosses were examined for independence were 331 and 257, respectively.

The percentage of F_2 eggs to reach the adult stage was lower than that

from the F_1 cross between Lausanne females and tu-h males. This also was attributed to differences in environmental conditions at the time the experiments were in progress. To check this explanation the reciprocal crosses were repeated in the same laboratory and as nearly as possible under the same conditions as those for the F_2 crosses summarized in table 2. The results of these crosses (series 1) are summarized in table 3. The percentage of eggs to reach the adult stage from the Lausanne X tu-h cross was comparable with the results from both F_2 crosses shown in table 2.

TABLE 3

Percentages of eggs to reach the adult stage in the F_1 from reciprocal crosses between tu-h and Lausanne S at 23° C and classification of adults for tumorous head character.

Series	Cross	Eggs collected	% eggs to reach adult stage	Adults				
				Abn. ♀	+♀	Abn. ♂	+♂	% Abn.
1	*tu-h × Lausanne	1250	42	71	190	38	207	21
	Lausanne × tu-h	875	66	5	251	6	273	2
2	tu-h × Lausanne	175	31	9	20	4	16	22
	Lausanne × tu-h	150	65	0	46	0	52	0

* Female parent is always written first.

The same reciprocal crosses were later repeated for a third time as nearly as possible under the same conditions. These results are also summarized in table 3 (series 2). The adult flies from the F_1 reciprocal crosses were classified for the expression of the tumorous head character. From the crosses between tu-h females and Lausanne males 21 and 22 percent of abnormal flies were obtained from series one and series two, respectively. This corresponds with expected results at 23°C based on previous experiments. From the reciprocal crosses two and 0 percent of abnormal flies were obtained. This also corresponds with expected results. These data show that the maternal effect was operating in the penetrance of the character, in the crosses designed to determine whether or not the maternal effect was also involved in the differential viability of the flies.

It has been shown that the tu-h stock is lower in viability than Oregon R and Lausanne S. A parallel with the maternal effect on penetrance has been observed on viability from the results of three series of reciprocal crosses. The relationship between the differential viability and the *tu-1* and *tu-3* genes is difficult to establish. Isogenic stocks would be desirable for this purpose. Since both *tu-1* and *tu-3* have no appreciable visible effect when alone it has been impossible to develop and maintain isogenic stocks known to carry these genes without introducing more variables into the experiment. The only method devised so far to isolate the *tu-1* and *tu-3* chromosomes makes use

of dominant markers. The markers are known to influence viability and their interactions vary in different combinations making difficult the selection of controls as a basis for comparison.

DISCUSSION

The penetrance and expressivity of the tumorous head character can be influenced markedly by genetic and environmental factors. A temperature effective period in the egg stage has been identified. Low temperature during the critical period is known to increase the length of the developmental period. Low temperature is associated with a low expression of the trait. High temperature and optimum nutrition decrease the length of the developmental period. An increase in penetrance and expressivity follow high temperature treatment during the critical period. This suggests the possibility of a relationship between the tumorous head expression and the rate at which basic reactions occur during the early developmental period.

Several authors have described a relationship between developmental rate and the expression of mutant characters. CHILD (1939) and BRAUN (1939) have shown that the expressions of certain wing mutants (members of the *vg* series of alleles) are influenced by the length of the developmental period. VILLEE (1945) has shown that the expression of aristapedia is changed in the presence of certain "growth rate" genes. DUNN and MOSSIGE (1937) have shown that the Minute mutants retard development and thus influence other characters.

The viability of the tu-h flies was shown to be lower than that of the controls at normal temperature and considerably lower at high temperature. TIMOFEEF-RESSOVSKY and others (review by DOBZHANSKY 1941) have shown that many mutants have an adverse effect on viability and that the environment influences the effectiveness of the mutants on the viability of the flies. The present study has also demonstrated abnormal sex ratios indicating differential viability between the sexes when 30°C temperature treatment was applied during the egg stage. The increased expressivity and penetrance at 30°C, increasing the degree of abnormality and the proportion of abnormal flies, is interpreted as a factor in viability.

DOBZHANSKY and SPASSKY (1944) have studied the influence of certain chromosomes from natural populations of *Drosophila pseudoobscura* on the viability of the flies under different environmental conditions. Genetic modifiers as well as environmental factors were found to influence the viability of the flies with various chromosomal combinations. The importance of such differential viability in natural selection was pointed out by these authors. The present study has shown that although the relative viability is decreased in the tu-h stock, the presence of one of the factors (*tu-1*) responsible for the tumorous head expression has no effect on the relative viability when alone at 21°C or 30°C. This gene is responsible for the maternal effect but acts only in the presence of another gene (*tu-3*). The maternal effect is in-

fluenced by temperature but the gene which controls the maternal effect has no influence on viability when alone at either high or normal temperature.

HARTUNG (1948) has compared the viability of two tumor bearing stocks (*st sr* and *bu tu*) with that of a wild stock of *Drosophila melanogaster* during the larval stage. He found that both tumor strains had a viability of less than 50 percent at 30°C. The tumor stocks were less viable than the controls. The reduced viability at the high temperature was accompanied by a reduction in tumor incidence. The viability of the tumor strains at 25°C and 20°C was not significantly different from that of the controls. In the present study, the tu-h stock was less viable at 21°C than the controls. A relative loss of about 20 percent occurred during the egg stage. This period coincides with the temperature effective period. An additional 10 percent loss, compared with that of the controls, occurred between the early larval and adult stage at 21°C. The relative loss was considerably greater at 30°C.

SUMMARY

The penetrance of the tumorous head abnormality was found to be influenced by genetic modifiers and environmental factors. The percentage of abnormal flies varied directly with temperature from 54 percent at 18°C to 93 percent at 30°C during the temperature effective period in the egg stage. The average penetrance was 79 percent at 25°C. A higher penetrance and expressivity was observed among the females than among the males.

Abnormal sex ratios favoring the males were observed at temperatures above 25°C. This was interpreted to indicate that the more extreme expressions (especially among females) had become lethals. The tu-h flies were generally less viable than the controls. The greatest loss at 21°C occurred during the egg stage. This developmental period coincided with the temperature effective period. A lower viability was also observed for tu-h flies given temperature treatment during the egg stage, later in development (during the early larval to adult period) than was observed for the controls. Increased temperature at the critical period influenced the viability of tu-h much more seriously than the controls. Results of outcrosses indicated a maternal effect for viability as well as for the visible expression of the tumorous head character. The gene (*tu-1*) which controls the maternal effect was found to have no influence on the viability of the flies when alone at 21°C or 30°C.

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ANTIGEN VARIATION IN PARAMECIUM AURELIA, VARIETY 1

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IN recent years the importance of the cytoplasm as a determinant of hereditary characters has become increasingly apparent. It has even been claimed that the cytoplasm contains genetic units as autonomous and specific as the nuclear genes, though such claims have been much disputed. Paramecium is exceptionally suitable for the investigation of this problem, and thanks to the work of SONNEBORN and his colleagues a considerable amount of information concerning the effect of cytoplasmic materials on certain characters of the organism, such as the "killers" and the antigens, has been obtained.

Investigation of these phenomena has hitherto been concentrated on the "B" group of varieties of *P. aurelia* (mainly variety 4), and the evidence at first seemed to show that cytoplasmic heredity was a feature of these varieties only. By contrast members of the "A" group (including variety 1) showed only Mendelian heredity.

The object of the present study was a more detailed analysis of the genes concerned with antigen formation in a number of stocks of *P. aurelia*. For this purpose, variety 1 was chosen, because some early observations of SONNEBORN (1943) had indicated a straightforward, Mendelian, segregation of antigen types in this variety. It was hoped that a more complete knowledge of the genic system would make it easier to place in its correct perspective the influence of the cytoplasm, about which considerable information is now to hand in variety 4. At the same time it was hoped to reconcile the apparent discrepancies in the behavior of the two groups of varieties, for, as SONNEBORN (1945) points out, it is hardly likely that two so nearly related groups of organisms would contain totally distinct hereditary mechanisms.

MATERIALS AND METHODS

Four stocks of *P. aurelia* were used in these studies. They were stocks 90, 60, 41 and 61, all belonging to variety 1 and mating freely together. They had been collected from various localities in N. America and were kindly supplied by DR. SONNEBORN.

Techniques for culturing, crossing, induction of autogamy and preparation of antisera were substantially the same as those previously described (SONNEBORN 1950a).

RESULTS

Variation within stocks

Previous work with variety 4 (SONNEBORN 1950b) had shown that each stock, derived from a single homozygous individual, was capable of giving rise to a series of antigen types, and that this intra-stock variation was caused by some mechanism other than the substitution of one gene by another. In variety 1, it was very soon evident that a similar system occurred. For example, an antiserum was prepared by injecting paramecia of stock 90 into a rabbit, and designated anti-90G. At a dilution of 1/1500 this antiserum was effective in immobilizing paramecia of the same type as those injected. A small proportion of paramecia of the same culture were however completely resistant to the immobilizing action of the antiserum. Cultures of these resistant animals were then grown, injected into a rabbit, and a new antiserum, anti-90D, prepared. This second antiserum immobilized paramecia of the second type (90D), but not those of the first type (90G). By similar procedures it was found that stock 90 could develop yet other antigen types. In table 1, the interactions between three commonly formed types (D, G, S) of stock 90 with the three corresponding antisera are shown.

TABLE 1

Immobilizing titres of 3 antisera of stock 90. The dilutions shown are those required to immobilize a few paramecia in 2 hrs. Dilutions below 1/50 are not considered.

Serum	Paramecium types		
	90D	90G	90S
Anti-90D	1/1500	....	....
Anti-90G	1/50	1/1500	....
Anti-90S	....	1/50	1/1500

The weak cross-reactions shown in certain combinations may be due to the presence of a small proportion of animals of undesired type in the injected material; or, alternatively, to the presence of a heterologous antigen at some site other than the surface of the paramecium (SONNEBORN, unpublished) or to some other cause. In general, cross-reactions between different types of the same stock were remarkably weak.

Effect of growth at varying temperatures

Paramecia of stock 90, when grown at 25°C, were predominantly of type G, but a small proportion of them were of types D or S. Growth at 29° or higher resulted in the production of predominantly type D animals, while at 18° type S could be produced. Transformation of one type to another as a result of changing temperature occurred in a regular manner, according to conditions. For example, a certain sample of 90G animals, when grown at 29° with excess food, transformed to 90D after approximately 50 fissions (or

ten days); if growth was at 33°, this transformation occurred after ten fissions (or two days); while growth at 36° produced transformation in less than five fissions (one day). The actual change of type from 90G to 90D was always quite sudden, occupying the time of only about 2 fissions, and during this short transitional phase the animals reacted simultaneously to both anti-90G and anti-90D sera. During the preceding period of growth at a new temperature, however, no perceptible change occurred in the 90G animals; they remained exactly the same as they had been when grown at 25°. The paramecia are thus able to maintain either one or other of these two antigens, but not both simultaneously.

The transformation from 90G to 90D was readily reversible by growing the 90D animals at 25° with excess food. Under these conditions 90D reverted to 90G in about 11 fissions (three days).

Variation between stocks

All the four stocks studied (together with 11 others not described here) behaved similarly, though not identically, in their response to varying temperatures. All produced a characteristic series of antigens. In all stocks the antigens formed at 29°–33° corresponded to a greater or less degree to type D of stock 90; those formed at 25° corresponded to type G of stock 90, and those formed at 18° corresponded to type S of stock 90. This regular correspondence between the three types formed by each of the four stocks was revealed by both serological relationships and genic relationships, as will be shown.

Data showing the serological relationship between corresponding types in different stocks are given in table 2. (Non-corresponding types showed little or no serological relationship.) It will be seen that some "corresponding" types were indistinguishable by the serological tests used, *e.g.*, 90S, 60S and 41S, or 61D and 90D; others were rather closely related yet clearly not identical, as shown by cross-reactions, *e.g.*, 60D and 41D; while some were so distinct that they showed practically no cross-reactions at all, *e.g.*, 61D and 60D, or 90G and 60G. For this reason, in a former publication (BEALE 1951), separate letters were applied to certain types. (The present 90D = the former 90Y, 90G = 90Z, 60D = 60W, and 60G = 60X.) The simplified nomenclature adopted here is based partly on the gene analysis, presented below, and partly on the homologies between certain of the D and G types found here in variety 1 with some of the D and G types already known in variety 4 (SONNEBORN, WHALLON and BEALE, unpublished).

Some types were much more easily obtained than others. For example, in stock 90, type 90G was invariably obtained by growth at 25°, but at 18° the type 90S was formed only sporadically and frequently transformed back to 90G. In order to obtain a pure culture of 90S it was necessary to grow the animals at 18° and kill unwanted 90G animals with homologous antiserum. In stock 41 however the situation was reversed. At 18° 41S was readily produced, but at 25° 41G appeared only occasionally and often transformed to 41S or 41D. Thus the *stability* of the corresponding types of different stocks

TABLE 2

Immobilizing titres of 12 antisera, each tested against four "corresponding" types of paramecia.

Serum		Paramecium types formed at 29°–33°			
No.	Description	90D	60D	41D	61D
45	anti-90D	1/1500	1/50	1/50	1/1500
72	anti-60D	....	1/1500	1/500	....
23	anti-41D	....	1/250	1/1000	....
60	anti-61D	1/1000	....	....	1/1000
		Paramecium types formed at 25°			
		90G	60G	41G	61G
64	anti-90G	1/1500	....	1/150	1/100
7	anti-60G	....	1/500	....	....
32	anti-41G	....	....	1/1500	1/500
66	anti-61G	1/50	....	1/500	1/1500
		Paramecium types formed at 18°			
		90S	60S	41S	61S
63	anti-90S	1/1500	1/1500	1/1500	....
50	anti-60S	1/1500	1/1500	1/1500	1/150
20	anti-41S	1/1500	1/1500	1/1500	1/50
54	anti-61S	1/50	1/50	1/50	1/1000

is by no means the same. It is difficult to specify exactly the stability, or the optimal conditions for a given type, but in general the following were found to be rather unstable or to require a narrow range of conditions: 90S, 60G, 41G, 61G. All the other types described were readily formed at the appropriate temperatures.

The *order* of the types with respect to temperature was however remarkably uniform, not only with the four stocks described here, but also with all other stocks examined.

Gene differences between stocks

Crosses were made between animals of each of the four stocks in various combinations, and the F_1 animals so obtained made to pass through autogamy. Segregation of antigenic types in the exautogamous F_2's was observed, and an analysis made of the gene differences between the four stocks. In all these experiments the parental cultures were first passed through autogamy to ensure their complete homozygosity.

F_1's. Hybrids between any two stocks were found to develop, in about the fifth cell generation after conjugation and thereafter, a mixture of antigens characteristic of the two parents, that is, any F_1 animal could be immobilized by two kinds of antibodies, one of which would affect only one parent and the second the other parent. The delay of approximately five fissions between

introduction of a new nucleus at conjugation and the manifestation of some of its genes, was presumably connected with the time necessary for the construction of a new macronucleus and the gradual dispersal of the products of the old macronucleus. A similar period of delay was found by SONNEBORN (1950c) for the manifestation of a gene distinguishing stock 29 from stock 51 of variety 4. Table 3 shows the antigens formed in various hybrids, grown at the three temperatures.

TABLE 3

Antigenic types of F_1 hybrids

Parent stocks	29°–33° group	25° group	18° group
90 × 60	90D + 60D	90G + 60G	S
90 × 41	90D + 41D	90G + 41G	S
90 × 61	D	90G + 61G	90S + 61S
60 × 41	60D + 41D	60G + 41G	S
60 × 61	60D + 61D	60G + 61G	60S + 61S
61 × 41	61D + 41D	61G + 41G	61S + 41S

These results are arranged for convenience in columns headed by the temperatures at which the types shown were most readily formed. Some of the types however were unstable (like some of the parents), and transformed into types more characteristic of other temperatures, *e.g.*, the F_1 between stocks 60 and 41 at 25°, shown as 60G + 41G, often transformed even at 25° to 60D + 41D.

The F_1's showed the following features: (1) Whenever two parental stocks could develop distinguishable antigens in a given temperature group, antigens characteristic of both parents could be detected in the F_1; but the immobilizing reactions between F_1 animals and one or other of the parental antisera were usually less strong than the immobilizing reactions between the parental animals themselves and their respective homologous antisera. It may therefore be concluded that the amount of any one antigen in the F_1 was less than in the parent, though since the F_1 contained two antigens the total amount may well have been the same. Frequently the F_1's resembled one parent much more closely than the other. (2) The F_1 animals never contained, except as a temporary condition during transformation, mixtures of antigens characteristic of different temperature groups, *e.g.*, the F_1 90 × 60 contained either 90G + 60G or 90D + 60D, but not 90G + 60D or 90D + 60G etc.

F_2's. The exautogamous F_2 families showed segregation of the antigen types characteristic of the parent stocks. For example, from the cross 90 × 60 there appeared in the F_2: at 18°—type S; at 25°—types 90G and 60G in a ratio of 1:1, and at 33°—types 90D and 60D in a ratio of 1:1. In a given temperature group, no antigenic types appeared which did not occur in one or other of the parent stocks, and when the parents differed in the antigens produced at a given temperature, the two types reappeared in the F_2 in equal numbers. The results are summarized in table 4.

TABLE 4

Segregation of antigen types in ex-autogamous F_2 families

Cross	29°–33° group			25° group			18° group		
90 × 60	90D	60D	Dead	90G	60G	Dead			
	38	36	176	95	82	7			
90 × 41	90D	41D	Dead	90G	41G	Dead			
	95	91	6	142	138	94			
60 × 41	60D	41D	Dead	60G	41G	Dead			
	98	104	65	57	47	193			
60 × 61	60D	61D	Dead	60G	61G	Dead	60S	61S	Dead
	111	112	206	36	36	21	28	27	37
61 × 41	61D	41D	Dead						
	138	142	89						

The wide fluctuations in proportions of "dead" clones shown in table 4 are due to the fact that some of the types could be determined at five fissions after autogamy, and where this was possible there was very little mortality, but others had to be grown for prolonged periods at certain temperatures in order to bring about transformation to the desired temperature group, and when this was done many clones died out. Such a high mortality in F_2's involving diverse stocks has been reported previously by SONNEBORN (unpublished) and DIPPELL (1950). Fortunately the ratios obtained here are not disturbed, even with 50 percent or higher mortality.

Each of the viable F_2 clones shown in table 4 was grown at various temperatures, and it was then found that they could show a recombination of parental characters. For example, in an F_2 from the cross 90 × 60, there appeared, not only the two parental classes (90G at 25°, 90D at 29°–33°; and 60G at 25°, 60D at 29°–33°), but also two new classes (90G at 25°, 60D at 29°–33°; and 60G at 25°, 90D at 29°–33°). The two new classes occurred in approximately the same numbers as the two non-recombination classes.

TABLE 5

F_2s showing recombination of two pairs of antigen types

Cross		Old classes		Recombination classes		Dead
90 × 60	Formed at 29°–33° →	90D	60D	90D	60D	
	Formed at 25° →	90G	60G	60G	90G	
		20	17	18	19	118
90 × 41	Formed at 29°–33° →	90D	41D	90D	41D	
	Formed at 25° →	90G	41G	41G	90G	
		31	39	53	48	213
60 × 41	Formed at 29°–33° →	60D	41D	60D	41D	
	Formed at 25° →	60G	41G	41G	60G	
		32	22	22	24	197

TABLE 6

F_2 from the cross 60 × 61, at three temperatures

	Old classes		Recombination classes						Dead
Formed at 29°–33° →	61D	60D	61D	61D	61D	60D	60D	60D	
Formed at 25° →	61G	60G	60G	61G	60G	61G	60G	61G	
Formed at 18° →	61S	60S	60S	60S	61S	61S	61S	60S	
	5	5	6	7	10	6	6	10	37

Data on this subject are presented in table 5. There are signs of significant deviations from the expected 1: 1 : 1 : 1 ratios, but this is not surprising in view of the high mortality and the instability of certain types.

A similar recombination of antigenic characters occurred in all the other F_2 families. Stocks 60 and 61 differ markedly in the antigens they produce in all three temperature groups. Consequently the F_2 derived from a cross between these two stocks permits the detection of eight classes, of which two are the old ones and six are new, recombination, classes. Such an F_2 was analyzed, and the results are shown in table 6. Though the numbers in each class are small, representatives of all eight classes were obtained, as shown.

The results set out in tables 4, 5 and 6 can best be interpreted according to the following genetical system. Each of the four stocks contains genes at three separate loci—*g*, *d* and *s*. Only one of these gene-loci is normally effective in controlling the phenotype at a given time. Genes at the *g* locus are manifest in paramecia grown at 25°, genes at the *d* locus at 29°–33°, and genes at the *s* locus at 18°. The different stocks each have their characteristic alleles at each of the three loci, as indicated in table 7.

Those alleles which at present cannot be distinguished, namely the *s* alleles of stocks 90, 60 and 41, and the *d* alleles of stocks 90 and 61 are provisionally written without suffixes. There are therefore at present four distinct alleles at the *g* locus (g^{90}, g^{60}, g^{41}, and g^{61}), three at the *d* locus (d^{60}, d^{41} and d), and two at the *s* locus (s^{61} and s). None of these genes is completely dominant over any of its alleles, though some are nearly so.

Data so far obtained show no indication of linkage between any of the three loci, though the numbers obtained would not reveal loose linkages.

TABLE 7

System of antigen-determining genes in four stocks.

Stock	Antigens			Genes
	29°–33°	25°	18°	
90	90D	90G	90S	$d\ g^{90}\ s$
60	60D	60G	60S	$d^{60}\ g^{60}\ s$
41	41D	41G	41S	$d^{41}\ g^{41}\ s$
61	61D	61G	61S	$d\ g^{61}\ s^{61}$

The role of the cytoplasm

As described previously, there may be a delay of 50 or more fissions between transference of a culture to a new temperature and the onset of transformation from one antigen type to another. The occurrence of this delay makes possible the crossing of types which are stable at different tempera-

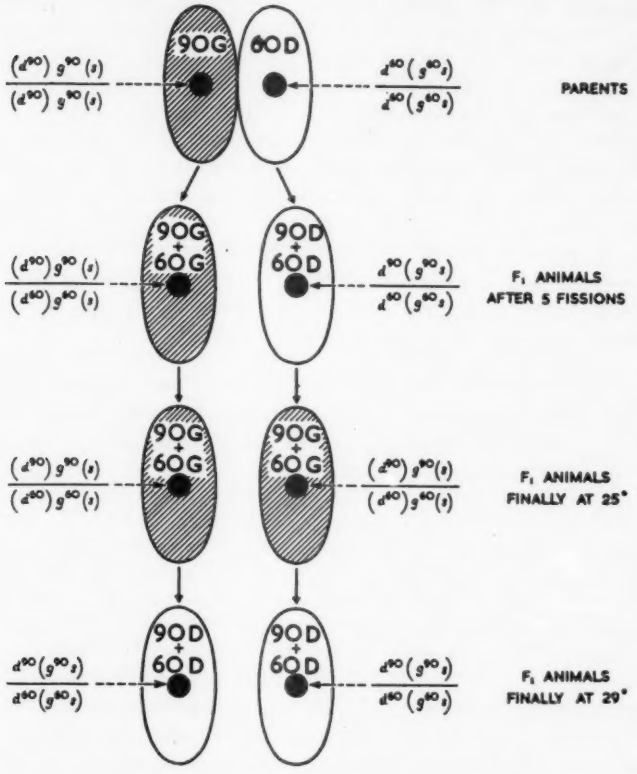

FIGURE 1.—The consequences of a cross between paramecia of the types 90G and 60D. The antigens are shown as large figures and letters and the genes as small italics. Genes which are not expressed are enclosed in brackets. Cytoplasm in the "25°" state is shown shaded; cytoplasm in the "29°" state is left clear.

tures, *e.g.*, 90G × 60D or 90D × 60G. Such crosses were made and the results are illustrated in figure 1. Considering the cross 90G × 60D it was found that after five fissions, those descendants which had received their cytoplasm from the 90G parent developed the antigens 90G + 60G, while those which had received their cytoplasm from the 60D parent developed the antigens 90D +

60D. If growth of the F_1 animals was continued at 29°, all the progeny finally came to have the antigens 90D + 60D, no matter what had been the parental cytoplasm; continued growth of the F_1 animals at 25°, however, eventually produced only 90G + 60G types.

These results show that the cytoplasm of the 90G animals is of such a nature as to permit the expression, not only of the gene g^{90}, but also of g^{60}. The cytoplasm of the 60D animals favors the expression of d^{90} and d^{60}. This property of the cytoplasm is transmitted for varying lengths of time to the descendants, but can be changed by varying the temperature.

It is therefore concluded that the action of varying temperatures on the expression of the genes at the three loci *g*, *d* and *s*, is effected through a modification of the cytoplasm. Considering again the cross 90G × 60D at 29°, it is to be noted that the nucleus from the 60D parent contains the gene g^{60} which is not expressed; but a nucleus from this parent migrates into the cytoplasm of the 90G parent and there contributes to the formation of a new macronucleus. The g^{60} genes in this new macronucleus are at once expressed, notwithstanding the fact that there has been no change of temperature, which is still 29°. What is necessary is that the cytoplasm into which the gene g^{60} is introduced should at some time previously have been brought to the "25°" state.

These conclusions have been confirmed by a number of other crosses. For example, 60G was crossed with 41D, and yielded, after five fissions at 25°, animals containing 60D + 41D if their cytoplasm was derived from the 41D parent, and 60G + 41G if their cytoplasm was derived from the 60G parent. In all such experiments it was found that the state of the cytoplasm determined which gene-loci were to be expressed, and that members of a series of alleles were favored by the same kind of cytoplasm.

Thus the earlier view of SONNEBORN (1948) that antigenic variations within a stock of variety 4 were cytoplasmically controlled, but that individual antigens could only be developed in the presence of particular genes, is strikingly confirmed here in variety 1.

DISCUSSION

From the preceding experiments, the following conclusions are drawn:

1. Each stock of *P. aurelia*, variety 1, contains a number of genes at different loci, corresponding to each of the antigens produced. Three such loci (*g*, *d*, *s*) have so far been identified. It is likely that there are several more.

2. Each stock has its characteristic alleles at each of these loci, though some alleles may be common to several stocks.

3. Within a homozygous stock only one immobilizing antigen is revealed at a time, except as a temporary phenomenon when a paramecium is transforming from one type to another. Consequently, genes at only one of the three loci can come to full expression at a given moment. There is a mutual exclusion of the products of non-allelic genes.

4. In heterozygotes, two antigens are regularly formed in the same animal, though each one separately in lesser amount than in the appropriate homozygote.

5. Which of the loci *d, g, s* will be expressed depends upon the state of the cytoplasm.

6. The state of the cytoplasm may be modified by varying the temperature of the external environment, though such modification need not occur immediately the organism is placed in the new environment. There may be a delay of 50 or more fissions. The change of state, however, when it does occur, takes place rapidly (two fissions).

7. The stability of the cytoplasmic states is a characteristic of each stock, and is presumably controlled by hereditary factors. The nature of these factors—whether they are the antigen-determining genes themselves, whether they are other, "modifying," genes, or whether they are some non-genic hereditary elements—is at present unknown. The result of this variation in stability of cytoplasmic states, however caused, is that certain antigen-determining genes are more readily expressed, under given conditions, than are their alleles in other stocks.

Much of the above theory conforms with that previously developed by SONNEBORN (1951 and earlier) for antigen determination in variety 4 of *P. aurelia*. The present work therefore affords no support for the early view (SONNEBORN 1945) that variety 1 made use of a genetical mechanism substantially different from that of variety 4, namely that differences in the "A" varieties (variety 1) were gene controlled only, while differences in the "B" varieties (variety 4) showed both genic and cytoplasmic control. We can now assert with confidence that both groups of varieties make use of essentially the same genetic system, involving both genes and cytoplasm. In variety 1, certain features of this system are now apparent which in variety 4 were less clear. They have been made clear as a result of the discovery that the antigen types formed at particular temperatures in one stock correspond both serologically and genetically with the antigen types formed at the same temperatures in other stocks. This has facilitated a more detailed genic analysis in variety 1, and led finally to the conclusion that cytoplasm which is in a state favorable for the expression of a particular gene, also favors the expression of alleles of that gene.

The earlier results of SONNEBORN (1943) and KIMBALL (1947) with variety 1 antigens can be readily accommodated in the theory adopted here. SONNEBORN obtained evidences of a single genic difference between stocks 1 and 60, from segregation of antigenic types derived from the two stocks. Similar segregations have many times been obtained in this work (see table 4). KIMBALL, using stock 60, by various treatments, including exposure to low temperature, was able to induce a transformation to "resistance" to a particular antiserum. It is now assumed that this transformation involved the substitution of a new antigen in place of the old (probably 60S in place of 60G), and not merely the loss of the old antigen, as thought by KIMBALL.

As regards the nature of the cytoplasmic materials responsible for production of the antigens, it must be admitted that we have at present very little information. It was formerly considered (SONNEBORN 1947b; SONNEBORN and BEALE 1949) that in variety 4 of *P. aurelia* the antigens were determined

by the activity of certain gene-like particles in the cytoplasm (plasmagenes). This was assumed because (1) antigenic variations within a stock were hereditary, persisting under certain conditions indefinitely, and (2) they were inherited through the cytoplasm. Subsequently, however, it was shown by SONNEBORN (1950b), that the specificity of some antigens was under strict gene control. For example, in variety 4, stocks 51 and 29 both produced a type designated A, though careful tests revealed a clear serological difference between the antigens 51A and 29A. Breeding tests showed that this difference was controlled by a single gene.

Furthermore, no evidence has yet been forthcoming to show the existence in the cytoplasm of any essential reproducible particles controlling the antigens. If a given antigen is replaced by another as a result of some change in the cytoplasm, the loss of the original antigen is never final; by changing the conditions it can always be recovered, provided only that the corresponding gene has not been replaced. There seem to be no elements in the cytoplasm which, once lost, cannot be recovered as a result of the activities of certain genes.

The new data presented here reinforce these doubts concerning the plasmagene type of hypothesis, in so far as the antigens are concerned. (The situation with regard to kappa is totally different, see SONNEBORN 1950b; BEALE 1951). Every antigen investigated in variety 1 has its specific controlling gene; variations in the state of the cytoplasm can be readily brought about, or reversed, by changing the temperature. We do not at present understand the cause of the variation in stability of the different cytoplasmic states, but that may also turn out to be genically controlled.

In order to prove the existence of autonomous cytoplasmic factors, it might be thought sufficient to demonstrate that two or more diverse kinds of cytoplasm could exist under identical genic and environmental conditions, that is, that variations in the cytoplasm could occur independently of variations in genes or environment. Such evidence was provided by the variety 4 antigen system, in which it was shown by SONNEBORN and LESUER (1948) that types A, B and D of stock 51 could be maintained indefinitely by growth at 26° with one fission per day. In variety 1 there are indications of similar phenomena. For example, animals of stock 90, grown at 29° with restricted food (sufficient for three fissions per day), remain for long periods either type 90G or 90D, whichever happens to be present at the beginning. Transformations from G to D and the reverse do however occur at rare intervals. Probably, under other conditions, complete stability of the two types could be obtained.

It has however been pointed out by DELBRÜCK (see SONNEBORN and BEALE 1949) that even in the variety 4 system, it is not necessary to postulate the existence of autonomous cytoplasmic factors, or as they are called, units "endowed with genetic continuity," in order to account for the observed behavior of the cytoplasm. It could be due to the operation of a flux equilibrium system capable of functioning in several ways under identical conditions.

We have to admit that there are at present no data which would permit us

to establish either the material nature of the cytoplasmic states, or what determines them. It is conceivable that they are determined by the activity of certain genes, whether connected with the antigen-determining system or not. On this view, a series of genes would liberate into the cytoplasm substances which would each correspond to a particular cytoplasmic state. There would be a competition between these substances, since only one cytoplasmic state is formed at any one moment, and the result of this competition would be decided, ultimately, by the external environment. Once established, a particular cytoplasmic state would persist at the expense of the other, competing, states through the operation of some such mechanisms as that postulated by DELBRÜCK. This is admittedly highly speculative and we must concede that a satisfactory solution of these problems awaits further experimental data. It is quite possible that some totally different explanation may turn out to be the correct one.

Whatever may be the true nature of the cytoplasmic elements responsible for these phenomena, their principal value lies in the model they provide for cellular differentiation in higher organisms, as SONNEBORN (1947b) has pointed out. Since most cells of a higher organism are presumed to contain sets of the same genes, differentiation is thought to occur through the development of diverse cytoplasms. The results of the experiments here described exemplify how such a mechanism might function. Certain cells in a developing organism are exposed to the action of a changing environment, which could bring about a change in the state of the cytoplasm. This in its turn would permit the expression of certain genes, and inhibit the expression of others, thus leading to the production of diverse cells. In the antigen system there is a limited number of sharply distinct cytoplasmic states (the number being limited by the number of gene-loci operating in the system) and this would parallel the occurrence, in cellular differentiation, of a number of sharply distinct types of cell. As WADDINGTON (1950) stresses, there is not an unlimited number of types of cell, with innumerable intermediates.

It has been possible to devise this model, not because the genetics of the *Paramecium aurelia* is in any fundamental way different from that of higher organisms, but simply because certain types of experiment are at present possible with Paramecium alone. This is due to two facts: (1) cells of Paramecium can be exposed to diverse environments, in such a way as to bring about long-lasting modifications, and (2) such modified cells can be caused to undergo conjugation, a process which permits a separation of nuclear from cytoplasmic factors.

SUMMARY

1. In *Paramecium aurelia*, variety 1, genes at three independent loci *g*, *d* and *s* have been shown to be concerned with antigen production. Each stock of paramecia contains characteristic alleles at each of these loci. So far, four alleles have been demonstrated at the *g* locus, three at the *d* locus and two at the *s* locus. The antigens corresponding to allelic genes usually show serological relationships.

2. Only genes at one of the three loci are normally expressed at a given time. Which one is expressed depends upon the state of the cytoplasm. There is a cytoplasmic state favorable for the expression of the *g* alleles, another cytoplasmic state favorable for the expression of the *d* alleles, and a third for the *s* alleles.

3. The state of the cytoplasm is determined to some extent by the temperature. At 29°–33°, the *d* alleles are normally expressed; at 25° the *g* alleles, and at 18° the *s* alleles. Modification of cytoplasmic state by change of temperature may be subject to prolonged delay, but the actual transformation from one state to another is a relatively sudden event.

4. The stability of corresponding cytoplasmic states under standard environmental conditions varies widely in different stocks.

5. The system of antigen-determination in variety 1 of *P. aurelia* does not differ in any essential respect from the variety 4 system previously described.

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ACTION OF THE BAR SERIES IN RELATION TO TEMPERATURE STUDIED BY MEANS OF MINUTE-N MOSAIC TECHNIQUE

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VARIOUS types of mosaics have been described from time to time in scientific literature, in both plants and animals. Mosaicism is generally attributed to a change in the genetic constitution of the organism in one of its embryonic cells. If this genetic change occurs at the time of the two-cell stage we then have the formation of an organism with one-half of the body of one genetic constitution and the other half of another. If the change occurs at the four-cell stage, a quarter of the organism will be mosaic. As the change in the genetic constitution occurs still later in the development of the individual the mosaic area will be correspondingly smaller. Theoretically, it is possible to have a mosaic area of only one cell. While this relationship between the time of the genetic change and the size of the mosaic area appears logical and simple, in the actual practice the relationship is much more complex.

Through the extensive work of STERN (1936) and other workers in this field, it is now generally accepted that the greater proportion of mosaics in Drosophila are a result of somatic crossing over. Earlier, STURTEVANT (1932) had suggested the use of mosaics as a means of studying the developmental effects of genes. Since the change in the genetic constitution may take place at any period during the development of the fly, the relative size of the mosaic area will be indicative of the relative time this genetic change occurs. Therefore, by properly synthesizing the desired genetic constitution of the fly, and by collecting a series of mosaics resulting among the females, we have then an excellent method of studying the action of a particular gene in relation to time and position. For example, if one wishes to study the action of the Bar gene, the first step would be to synthesize female flies with w1/+B genetic constitution. When somatic crossing over occurs in one of the cells of the embryonic eye, the two subsequent daughter cells will have a different genetic composition from the original synthesis. One daughter cell will be w1/w1; the other, +B/+B. The former will be expressed as white and non-Bar (wild) facetted-tissue, the latter as red homozygous Bar facetted-tissue. These two types of tissues will lie side by side along the original heterozygous red and Bar facetted-tissue. The w+/w+ tissue is readily recognized as a patch of white facets; the +B/+B tissue is difficult to identify since it appears similar to the w+/+B tissue.

Here then, we have a non-Bar tissue, w+/w+, or cells whose genetic constitution is for a large, wild type eye growing along the side of a tissue whose genetic composition is for a smaller eye, w+/+B and +B/+B. This affords the

worker an excellent technique for the study of some of the basic problems of genic action and its relationship to embryonic induction and cell differentiation.

This simple procedure has three advantages over the transplantation technique. One, it avoids mechanical factors or disturbances that are usually involved in transplantation technique. Two, the tissue is studied in the region of the organism where the genetic factors in question primarily express themselves. Three, a small part of the tissue may be studied at a time. For example, it is very difficult to transplant a small piece of the optic disc from one larva into the optic disc of another larva. Moreover, the use of mosaics in studying genic action has the distinct advantage over the temperature-effective-period technique in that no secondary thermal effects are involved during the developmental period. It is also superior to the X-ray technique in that the problem of randomness of effect is reduced considerably. When all the experimental advantages and disadvantages are considered, the mosaic technique lends itself best to the study of the problems of genic action in relation to embryonic induction and cell differentiation.

The object of this experiment is to use mosaics of the Bar series for the study of genic action and the way the latter is related to embryonic induction and cell differentiation.

PROCEDURE

Females with the following genetic composition were synthesized; M-n/wfBB, +/wfBB, M-n/wfBiBi, +/wfBiBi, M-n/wfB, +/wfB, yBiBiM-n/w, yBiBi+/w. These were obtained from the following crosses; M-n/+ ♀ × wfBB ♂, M-n/+ ♀ × wfBiBi ♂, M-n/+ ♀ × wfB ♂, yBiBiM-n/wBiBi+ ♀ × w ♂. The different members of the Bar series, Bar (B), double Bar (BB), double infra Bar (BiBi), were used to study the role these factors have in reducing the size of the eye. The Minute-n (M-n) was used to increase the frequency of the mosaics. In addition, this gene produces lethals in homozygous state, and reduces the size of the bristles in the heterozygous condition. The white gene (w) was added as a marker to distinguish the difference between heterozygous Bar facetted-tissue and wild type facetted-tissue, and heterozygous Bar and homozygous Bar facetted-tissue. The forked-gene (f) modifies the shape of the bristles over the head and body. It was used to distinguish mosaic areas occurring over these parts. The yellow gene (y) was substituted for the forked gene as a marker for body mosaics.

Each female resulting from the above crosses was observed for mosaic condition occurring in the eyes, head ocelli, legs, thorax, and abdomen. Every eye mosaic found was recorded by taking a photomicrograph of it. This method of recording has been found very effective in studying this type of data. It is accurate and permanent. By this method innumerable details which normally escape detection by the observer are recorded and can be used in some future time in evaluating the data. The data obtained and described in this experiment were taken from over one hundred of these photomicro-

graphs, and from direct observations. The apparatus used in photomicrographing the specimens consisted of a simple arrangement of a compound microscope with a fixed vertical camera attached at the end of the ocular piece of the microscope. A bright vertical tungsten illumination was used as a source of light. Most of the photographs were taken at a magnification of 100×, and in black and white.

The head and body mosaics were recorded by a different technique. Each mosaic of this group was recorded on a semi-diagrammatic outline of the fly especially prepared for this purpose (see fig. 1). The eye mosaics also were kept on this form. These will be described a little later on in the data.

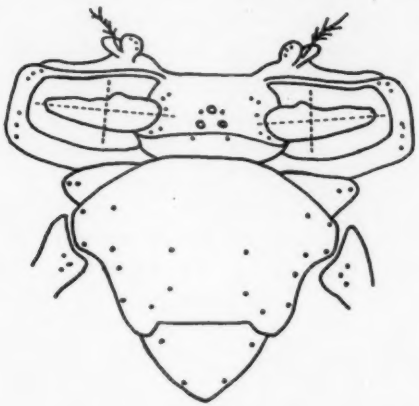

FIGURE 1.—A semi-diagrammatic drawing of the fly used to record position and extent of the mosaic areas occurring in the eye, head, thorax and fore-legs. This represents a dorsal view, showing head, thorax and part of the fore-legs. The sides of the head have been conveniently spread out to record the eye mosaics. The eyes are arbitrarily divided into four quadrants; anterior dorsal, anterior ventral, posterior dorsal, posterior ventral regions. The dots represent the location of all the major bristles affected.

The above synthesized flies were studied at two different temperatures, 20 ± 0.5 and 25 ± 0.5°C. The food medium consisted of banana-agar mixture placed in shell vials, 25 × 95 mm in size.

DATA

Frequency of mosaics

A total number of 49,211 female flies were investigated, of which 597 were mosaics. These included mosaics of the eye, head, thorax and abdomen. Tables 1 and 2 summarize the data obtained on the frequency of mosaics at 20 and 25°C. Column 1 lists all the different genetic constitutions of the females synthesized for the purpose of this investigation. The data listed in these two tables have been arbitrarily divided into two parts; flies containing

Minute-n gene which are referred to simply as Minutes, and flies with the wild type allelomorph which are designated as non-Minutes. The various types of mosaics have been classified into two main groups, those involving the body and head are in column 3 and those affecting the eye are in column 5. The respective calculated percentages are included in columns 4 and 6.

It is apparent from tables 1 and 2 that the percent frequency is considerably higher among the Minutes than in the non-Minutes. Just as BRIDGES (1925) first found and as others have found since these data confirm the general validity that the addition of the Minute-n gene increases the frequency of mosaics. In this sense these data add nothing new to the knowledge of the

TABLE 1

Frequencies of Minute-n and non-Minute-n mosaics of the Bar series, developing at 20° C.

Minute females	Number of non-mosaics	Number of body and head mosaics	Percent frequency body and head	Number of eye mosaics	Percent frequency eye mosaics
M-n/wfBB	2530	40	1.55 ± 0.243	12	0.47 ± 0.135
M-n/wfBiBi	2755	242	8.00 ± 0.493	30	0.99 ± 0.180
M-n/wfB	2271	3	0.13 ± 0.076	22	0.96 ± 0.204
yBiBiM-n/w	1716	1	0.06 ± ?	24	1.38 ± 0.279
fBM-n/w	2155	4	0.18 ± 0.092	22	1.01 ± 0.214
Total	11427	290	2.45 ± 0.142	110	0.93 ± 0.088
non-Minute females					
+/wfBB	2914	....	....	2	0.07 ± ?
+/wfBiBi	3273	....	....	1	0.03 ± ?
+/wfB	2947	....	....	4	0.14 ± 0.068
yBiBi+/w	2136	....	....	1	0.05 ± ?
fB+/w	2835	....	....	5	0.18 ± 0.079
Total	14105	....	....	13	0.09 ± 0.026

Minute-n gene. However, the data listed in these two tables with respect to temperature effect on the frequency of mosaics and the non-Minute mosaics reveal some interesting results. In table 1 we notice that the total percent frequency for the Minute eye mosaics is significantly higher than that of the non-Minutes, a calculated difference of $x/\sigma = 9.15$. In table 2 the percent frequency is also significantly higher for the Minute eye mosaics than the non-Minute mosaics, with a calculated difference of $x/\sigma = 3.91$. There is also a significant increase in the percent frequency of the Minute eye mosaics in flies raised at 20°C than those raised at 25°C, $x/\sigma = 2.74$. The opposite results are obtained when we compare the non-Minute eye mosaics at these two temperatures. The percent frequency is significantly higher at 25°C than at

20°C in which the calculated difference is $x/\sigma = 3.22$. Comparing the percent frequency of the body and head mosaics with the percent frequency of the eye mosaics for the Minutes at 25°C in table 2, we find no significant difference. It is difficult to make a comparison between these same two groups of mosaics raised at 20° C found in table 1, since series M-n/wfBiBi gave an abnormally high percentage of body mosaics. An attempt was made to obtain a stock of this high frequency mosaic-producing variety but it was unsuccessful.

STERN and RENTSCHLER (1936) found no significant difference in the frequency of mosaics in flies raised at 17°C and those at 25°C. These results

TABLE 2

Frequencies of Minute-n and non-Minute-n mosaics of the Bar series, developing at 25° C.

Minute females	Number of non-mosaics	Number of body and head mosaics	Percent frequency body and head mosaics	Number of eye mosaics	Percent frequency eye mosaics
M-n/wfBB	3353	26	0.77 ± 0.150	13	0.38 ± 0.106
M-n/wfBiBi	287	10	3.24 ± 1.010	11	3.57 ± 1.057
M-n/wfB	4714	43	0.90 ± 0.137	22	0.46 ± 0.098
yBiBiM-n/w	1777	0	0	17	0.95 ± 0.229
fBM-n/w* (at 30° C)	417	1	0.24 ± ?	4	0.95 ± 0.149
Total	10131	79	0.77 ± 0.086	63	0.61 ± 0.077
non-Minute females					
+/wfBB	4009	....	....	9	0.22 ± 0.075
+/wfBiBi	356	....	....	1	0.28 ± ?
+/wfB	5628	....	....	15	0.27 ± 0.067
yBiBi+/w	1970	....	....	6	0.30 ± 0.124
fB+/w* (at 30° C)	571	....	....	6	1.04 ± 0.422
Total	11963	....	....	31	0.26 ± 0.046

*This series has not been included in the total.

seem contrary to those found above. The difference in these results could be attributed to several factors, such as temperature (difference between 17° and 20°) and the genetic composition (presence of sn^3 in one case and Bar in the other, or other modifying factors peculiar to different stocks).

At the time this experiment was undertaken no attempt had been made to collect non-Minute body mosaics. Therefore, portions of the tables that are marked in dashes indicate that no effort had been made to collect the specimens, while those marked with a zero show that an effort had been made but none were found. No comparison can be made between the percent frequency of the Minute and non-Minute body and head mosaics. The data for fBM-n/

w series at 30°C have been included in table 2, but have not been entered in the calculations.

Sizes of mosaic areas

The sizes of the mosaic areas varied considerably. In the case of the eye the areas ranged from a single white facet to a complete white wild type eye (approximately 800 facets). The quantitative aspect of these eye mosaics has already been discussed elsewhere (DEMARINIS and HERSH 1943) and will not be reviewed here. In the case of the head and body mosaics, which are easily identified by forked bristles, the areas ranged from a single forked bristle to an area involving the complete head and thorax. The greatest number of mosaics found in this experiment were within these two limits. Body mosaics were further identified by the presence of a sex comb. Nineteen cases were recognized in this manner. Other body mosaics, especially those involving the abdomen, were identified by the characteristic appearance of the male tergites. Nine such cases were found. In some of these only a small portion of one tergite was affected; in others the area extended over one complete half of the abdomen. Mosaic areas occurring on the dorsal portion of the head were further identified by the change in the pigmentation of the ocelli, from red to white. Thirty-nine specimens were found in which the ocelli were affected. Some of these involved one ocellus, some two, and others all three ocelli. The wing was also used as a means of identifying mosaics. The heterozygous M-n wing is considerably shorter than that of the wild type female but definitely longer than the wing of the wild type male. Eighteen mosaics were found in which the wing was involved. This group also includes the gynandromorphs which are listed in table 4. This gives us a total of 46 body mosaics which have been identified as male areas. These are best explained on the basis of the X-chromosome elimination rather than somatic crossing over. STERN (1936) was able to show another possible chromosomal mechanism to explain male area. However, until definite cytological evidences are presented, the present discussion will use the simple X-chromosomal elimination hypothesis to explain male areas.

Relative sizes and positions of mosaic areas in the eye

Particular attention was given in analyzing the different eye mosaics as to sizes and positions in the eye. The relative size of the affected area as used here refers to a general description of size, such as large, intermediate or small. The exact sizes in terms of facet-number have been given in a previous report (DEMARINIS and HERSH 1943). In carrying out this study, a semi-diagrammatic outline of the dorsal view of the fly was made, with the left and right sides of the head spread out in the same plane as the dorsal view of the head and thorax (see fig. 1). The outline of the eye, a typical heterozygous Bar eye, was arbitrarily divided into four quadrants, dividing the eye into anterior and posterior halves and into dorsal and ventral halves. Dividing the eye into four quadrants enables one to tabulate the number of times

the white areas appeared in each of the four quadrants. This is nothing more than a technique of systematizing the data. (The results of this tabulation are given in table 3.) This outline was used to mark the location and extent of affected areas in the eyes, and also to indicate the head and body mosaics. This was done simply by marking the exact bristles affected. Each mosaic was recorded on a separate outline. A combination of photomicrographs and outlines such as these enabled the observer to keep a very accurate record of the data on each mosaic.

Table 3 lists the different genetic constitutions of the females studied, and the number of times the white area appeared in each of the four quadrants, anterior dorsal, anterior ventral, posterior dorsal, and posterior ventral. The two temperatures for each group are also indicated. Here we note that the mosaic areas appear more frequently on the anterior side of the eye than on

TABLE 3

Eye Mosaics. The number of times the white area appears in each of the four sections of the eye, Anterior Dorsal, Anterior Ventral, Posterior Dorsal, and Posterior Ventral for the different types of mosaics raised at 20° C and at 25° C.

Genetic constitution of females	Temperature	Anterior dorsal	Anterior ventral	Posterior dorsal	Posterior ventral
M-n/wfBB	20° C	7	1	2	0
	25° C	7	0	8	8
M-n/wfBiBi	20° C	17	2	10	3
	25° C	2	2	4	4
M-n/wfB	20° C	9	7	8	2
	25° C	17	8	7	9
yBiBiM-n/w	20° C	10	8	6	0
	25° C	11	5	4	2
Total number of times		80	33	49	28

the posterior side, 113 times as compared to 77 times, or in the ratio of approximately 3 : 2. On the basis of the theory that the mosaic areas occur with equal frequencies in the posterior section and the anterior section of the eye, chi-square computation indicates that the observed difference is significant, chi-square = 4.93. Likewise, the areas occur more frequently in the dorsal lobe than in the ventral lobe, 129 times as compared to 61 times, or a ratio of approximately 2 : 1. Calculation gives chi-square = 24.4. This value indicates that the observed ratio is significantly different from the theoretical ratio. Also, it is quite apparent that the higher frequency of mosaic areas occurring in the anterior dorsal region of the eye is significantly different from the theoretical, chi-square = 44.4. These data seem to suggest one of two things; either that the genetic change occurs more frequently in the cells located on the anterior region of the embryonic eye or that (if we assume the genetic change is constant and can occur as frequently in one cell as in another) there is a

proportionately greater number of embryonic cells on the anterior portion of the eye disc which contributes to the adult eye. The latter suggestion seems plausible in view of the following additional observations made on these mosaics. It is interesting to note that STERN (1936) found spotting to occur more frequently on the abdomen than on the thorax and head. The difference in the frequency of spotting can also be interpreted on the basis of the relationship between cell-number and frequency of genetic change occurring at different stages of development.

In all cases where the mosaic area consisted of approximately ten facets or more and appeared on the anterior boundary of the eye the typical outline of the heterozygous eye became definitely modified in that region. When the genetic change is for a wild size eye (w+/w+) the normal outline of the anterior section of the heterozygous eye bulges out (see photomicrograph c and d in fig. 2). The normal outline recedes in that same portion of the eye, in cases where the genetic change is for a homozygous Bar (wB/wB) (see photomicrograph a, fig. 2). On the other hand, white areas of similar sizes and of similar genetic constitutions appearing on the posterior border of the eye do not distort the posterior outline (see photomicrograph b, fig. 2). Here the rows of white facets are continuous with the rows of red facets, and the contour of the posterior border remains unchanged from that of a typical heterozygous eye. A genetically different facetted-tissue for eye size, whether it be for a larger eye (w+/w+) or a smaller eye (wB/wB), does not seem to express itself as such in either the dorsal or ventral posterior region of the eye. These same genetically contrasting tissues express themselves very markedly in the anterior dorsal and ventral regions.

Head mosaics

The mosaic areas in the head regions are readily identified by the forked type of bristle. Like the eye mosaics, these can be explained on the basis of either somatic crossing over, forming tissue with (f/f) genetic constitution, or on the basis of X-chromosome elimination which results in the formation of tissue with (f) genetic constitution. Since the forked gene is very close to the Bar locus, it was not surprising to find a large number of eye mosaics involving circumocular bristles. The sizes and positions of these forked areas varied considerably. They ranged from an area with a single forked bristle to one involving the entire head. No particular region of the head was affected more than another. When the affected area involved the ocellar bristles, the ocelli themselves were white instead of the normal red. In this experiment, however, particular attention was paid to small areas involving both facetted and non-facetted tissues at the same time. Forty-seven of these mosaics were found in which the affected area involved one to four white facets adjacent to the non-facetted tissue containing one forked circumocular bristle. The smallest identifiable area of this type was one white facet immediately adjacent to one forked circumocular bristle. Twenty-nine of the forty-seven were one to

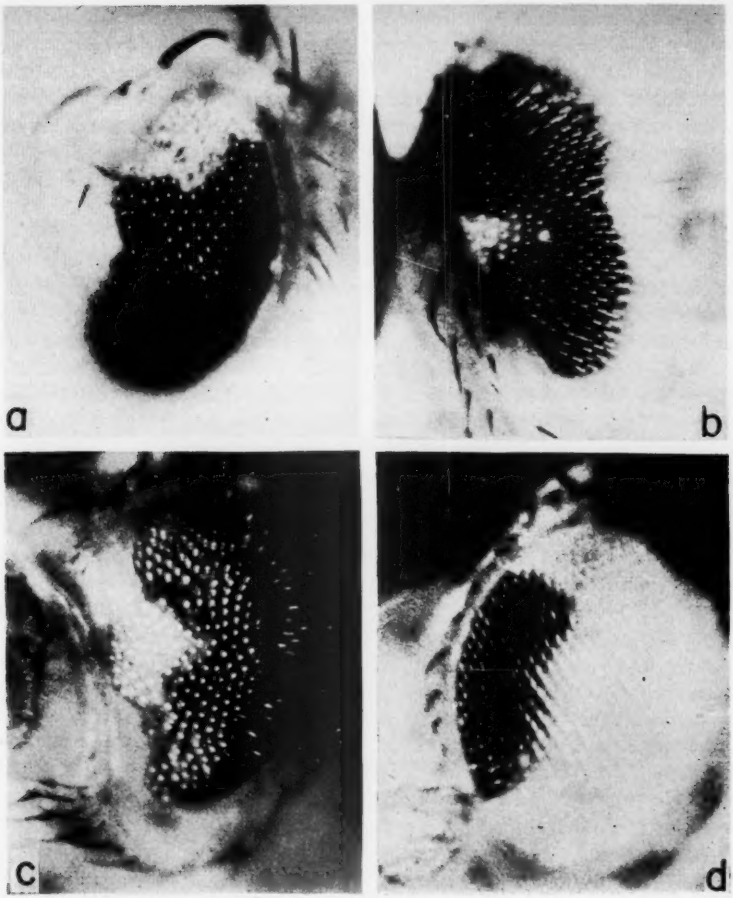

FIGURE 2.—Photomicrographs of the Bar-eyed mosaics. (a) M-n/wfB type of fly with a wB/wB area in the anterior dorsal region of the eye. Note the recession of the boundary line in that region. (b) M-n/wfB type of fly with a wB/wB area in the posterior region of the eye. There is no evidence of recession of the posterior boundary line in that region. (c) fBM-n/w type of fly with a w/w area on the anterior section of the eye between the dorsal and ventral lobes. Observe the bulging of this area anteriorly. (d) fBM-n/w type of fly with a w/w area on the anterior region of the eye. In this specimen the genetic change has occurred relatively early in development and the bulging is proportionately greater, practically completing the pattern of the wild type eye (544 total facets). Note also the outline and position of the dark area (red-facetted area, fBM-n/w). It appears similar to a heterozygous Bar eye. (See discussion and fig. 3 for the possible theoretical interpretation of these data.)

one type (one white facet adjacent to one forked bristle). These small areas indicate that the event of the genetic change must have occurred relatively late in development. Moreover, it indicates that the differentiation of cells, those which ultimately form the white facet, and those which form the forked bristle, occurs very late in development. This interpretation assumes the hypothesis that each mosaic area arises from a single embryonic cell of the optic disc.

Gynandromorphs and other less common types of mosaics

One generally considers gynandromorphs as organisms having both male and female characteristics. The bilateral and antero-posterior types of gynandromorphs occur infrequently. Even rarer in occurrence are the bilateral and antero-posterior mosaics (those which are completely of one sex but have one-half of the body of one genetic constitution and the other half of another). These are nearly always females. The following descriptions of these less common types, while not entirely new, serve to complete the presentation of the data. (See table 4.) Five antero-posterior gynandromorphs were found in which the anterior half of the fly was male and the posterior half, female. The main guiding characteristics used in identifying male and female parts were the sex combs, size of wings, and the appearance of the tergites of the abdomen. None of these antero-posterior specimens had a common line of demarcation between the anterior and posterior halves. In one specimen the front half involved the head which had white bar eyes, forked bristles, white ocelli, and the front third of the thorax which was covered by forked bristles, and the fore legs with sex combs. The remaining posterior two-thirds of the thorax was covered by straight bristles and the abdomen was typically female. In another specimen the anterior portion also had the characteristics just described, except that the thorax was completely forked. In still another, the complete thorax and head were affected except for a few red facets in the posterior section of each eye. In all of these gynandromorphs there seemed to be no common basic pattern. Even among the so-called bilateral types of gynandromorphs only a few are true bilateral, and these have been listed in table 4. The intermediate types, that is, those which fell short of the typical bilateral or antero-posterior mosaics have not been listed in table 4, but in table 1 under the general group of body and head mosaics.

All of the antero-posterior gynandromorphs listed in table 4 were mated and were found fertile. They reproduced according to the original genetic constitution of the zygote.

A significant fact derived from these observations is that nine were male-female type and only two were complete females. Since male areas are best explained on the basis of the X-chromosome elimination, these data show that the X-chromosome elimination is much more common than crossing over among the early cleavage nuclei. Only two of the gynandromorphs listed in table 4 can be explained on the basis of double crossing over occurring between the Bar locus and M-n, and M-n and the spindle attachment.

TABLE 4

Gynandromorphs.

Original genetic constitution of zygote	Type of gynanadromorph	Descriptive
M-n/wfBB	antero-posterior (X-chromosome elimination)	anterior ♂ wfBB, head, thorax, legs / posterior ♀ abdomen
M-n/wfBB	bilateral (X-chromosome elimination)	left ♀ M-n/wfBB / right ♂ wfBB
M-n/wfBB	bilateral (Double crossing over)	left ♀ M-n/wfBB / right ♀ +/wfBB
M-n/wfBB	bilateral (X-chromosome elimination)	left ♂ wfBB head; ♀ abdomen / right ♂ wfBB head, thorax; ♀ abdomen
M-n/wfBiBi	bilateral (Double crossing over)	left ♀ M-n/wfBiBi / right ♀ +/wfBiBi
M-n/wfBiBi	bilateral (X-chromosome elimination)	left ♂ +/++++ / right ♀ M-n/wfBiBi
M-n/wfBiBi	antero-posterior (X-chromosome elimination)	anterior ♂ wfBiBi; head, fore part of thorax, legs / posterior ♀ M-n/wfBiBi; thorax, abdomen
M-n/wfB	antero-posterior (X-chromosome elimination)	anterior ♂ wfB; head, fore part of thorax, legs / posterior ♀ M-n/wfB, thorax, abdomen
yBiBiM-n/w	antero-posterior (X-chromosome elimination)	anterior ♂ w; one eye partly red / posterior ♀ yBiBiM-n/w; abdomen
yBiBiM-n/w	antero-posterior (X-chromosome elimination)	anterior ♂ w; head, thorax / posterior ♀ yBiBiM-n/w; abdomen
fBM-n/w	bilateral (X-chromosome elimination)	left ♂ w / right ♀ fBM-n/w

DISCUSSION

The data presented here show that the white areas occur more frequently on the anterior than on the posterior region of the eye, and that they occur even more frequently on the anterior dorsal position than on any other position on the eye. The interpretation of this is as follows: If we assume the hypothesis that each mosaic area is derived from a single event, that is, either by X-chromosome elimination or somatic crossing over, and that this event can occur just as frequently in one cell as another in the eye anlage, then the conclusion is that the anterior portion of the eye anlage contributes a proportionately greater number of cells in determining the final size of the eye. The larger number of embryonic cells involved in this region of the eye anlage may be accounted for in one of two ways; either by the increased rate of cell division, or by the prolongation of the period of cell division. In either

case, the indication is that the anterior portion of the eye anlage plays the prominent role in determining the difference between eye sizes of the Bar series.

The above interpretation (that the difference in eye sizes is actively determined by the anterior portion of the eye anlage) is further substantiated by additional observations on the relative sizes, positions and shapes of the white areas. It has been indicated previously that the mosaic areas appearing on the anterior region of the eye always distort the boundary line of the typical heterozygous Bar eye. The boundary line tends to shrink or recede posteriorly when the genetic constitution of the area is for a smaller eye (wB/wB). The boundary line tends to bulge or expand anteriorly when the genetic constitution of the area is for a larger eye (w+/w+). However, no distortions are observed when either of these two same types of tissues are located on the posterior part of the eye.

Let us first consider the case where the genetic constitution of the white area is wB/wB. This type of tissue will normally produce fewer facets than the adjacent wB/++ tissue. The reduction in the facet-number by wB/wB tissue is manifested by a recession of the anterior boundary line in that part of the eye. The recession occurs only on the anterior boundary of the area. This reduction in facet-number is a reflection of a reduction of the embryonic cells of the eye anlage, in that particular region of the eye. Now let us take the case where this same type of tissue (wB/wB) occurs in the posterior portion of the eye. Here we find no distortion of the eye. The posterior border does not recede in the same manner as it did on the anterior side. The rows of white facets are continuous with the rows of red facets. There is no apparent reduction of facet-number when wB/wB tissue is located in the posterior section of the eye as evidenced by the absence of any recession. Now let us consider the case where the genetic constitution of the white area is w+/w+ and is located on the anterior side. This type of tissue will normally form more facets than the adjacent wB/++ tissue. The increase of facet-number by w+/w+ tissue is manifested by a bulging of the anterior border in this particular section of the eye. However, when this same tissue occurs in the posterior region we notice no bulging or distortion of any kind. The white facets are continuous with the red ones. In short then, wB/wB and w+/w+ tissues react according to their genetic constitution when they are located on the anterior section of the eye, but not when located in the posterior section. In terms of genic action these observations seem to indicate that the Bar-factor controls the embryonic cell division primarily on the anterior section of the eye anlage. The Bar quantitatively reduces the size of the wild type eye by specifically reducing the number of cell divisions on the anterior portion of the eye anlage. Although we customarily think of the Bar as a factor for reducing the size of the wild type eye as a whole, the data presented here seem to indicate that the Bar-factor limits its expression to only a portion of the eye.

This deduction could be tested further if it were technically possible to transplant a small piece of wB/wB embryonic eye tissue on the anterior sec-

tion of a +/+ type eye anlage and another small piece in the posterior position. If the above deduction is sound, the anterior piece would produce fewer facets and distort the boundary line of the eye, while the posterior piece would form a relatively larger number of facets or equal to +/+ type of tissue of the host's eye. Unfortunately, our present-day technique of transplantation is too limited to carry out such an experiment.

The above interpretation of the data is in total harmony with our previous quantitative studies of the Bar series (DEMARINIS and HERSH 1943) and with GOLDSCHMIDT's (1945) hypothesis on gene dosage. One further modification needs to be made on the latter in order to bring it in line with the present observations and interpretation. Instead of thinking of the Bar dosage as reducing the eye anlage as a whole, it should be pictured as quantitatively affecting a definite number of embryonic cells on the anterior portion of the eye anlage. In the light of these new data, Goldschmidt's gene dosage hypothesis would be best illustrated by the hypothetical diagram given in figure 3. In this diagram we note that the Bar series gene dosage, starting with the wild type (+/+) and ending with homozygous double Bar (BB/BB), progressively reduces the eye from the anterior to the posterior. The effect is proportionately greater on the anterior dorsal lobe than any other part. The BB/BB-type of eye as well as other types do not actually appear in the position as illustrated in this diagram, but rather a considerable distance away from the posterior circumocular bristles. This difference in position may be easily accounted for by a mechanical shifting of tissues that may occur during development.

If we are correct in assuming that each mosaic area is derived from a single event, that is, either somatic crossing over by the X-chromosome elimination occurring in one of the embryonic cells, it seems clear then that both the circumocular forked bristle and the white facet originate from the same embryonic cell. In view of the fact that the event has occurred relatively late in development, it signifies that the differentiation of the two types of tissues must occur relatively late also. In short, the differentiation of facetted and non-facetted tissue takes place relatively late in development. This interpretation is well in accord with the observations made by BODENSTEIN (1938) and by STEINBERG (1941). Mosaic areas consisting of a single white facet also indicate that at least one of the steps in the whole chain of biochemical reactions is strictly intracellular in nature. This interpretation is likewise in agreement with the work of KIKKAWA (1941). While these conclusions merely confirm the work of others, they serve to demonstrate that the mosaic technique may be used as an experimental approach to obtain this type of information.

In view of the predominance of male-female type of mosaics found in this experiment (table 4) the indication is that the X-chromosome elimination occurs much more frequently than somatic crossing over in the early cleavage nuclei. The X-chromosome elimination also occurs in the later stages of development, as was evidenced by the female flies with sex combs, or shorter

wings, or male type tergites. The other mosaics found in this experiment could be equally well explained on the basis of either the X-chromosome elimination or by somatic crossing over. For example, one cannot distinguish a white area in the eye that is due to w/w or w, the former brought about by somatic crossing over, the latter by X-chromosome elimination. The same is true for the body mosaics where only the bristles are involved. One cannot distinguish the difference between f/f and f type of bristle. We had no way

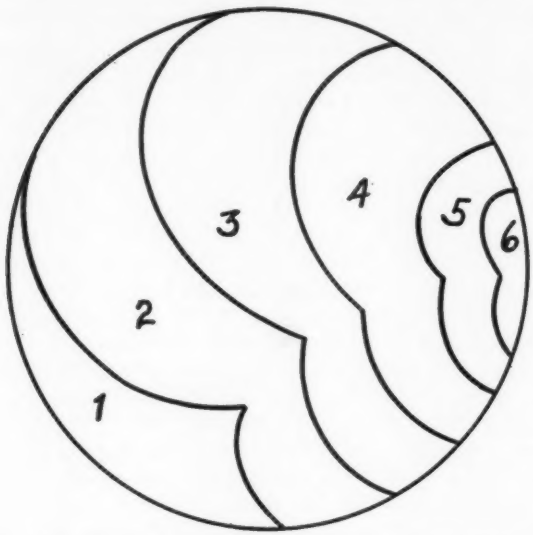

FIGURE 3.—A hypothetical diagram illustrating the relationship between gene dosage and eye size of the Bar series. (1) Represents +/+ type eye, which in this case covers the entire area of the circle. (2) Represents +/Bi type of eye, showing a proportionate reduction of facets on the anterior region. (3) +/B type eye with a further reduction of facets on the anterior portion. (4) +/BB type eye. (5) B/BB type. (6) BB/BB type. The eye becomes progressively smaller from the anterior to the posterior with each additional Bar factor. See also figure 2, photomicrograph *d*, and note the position of the heterozygous Bar area (dark) in relation to the wild type area (white). The various eyes of the Bar series do not actually appear in the same position as illustrated in this hypothetical diagram. A mechanical shifting of tissue occurring during development may account for this difference.

of determining how many mosaics were due to X-chromosome elimination and how many were due to somatic crossing over. It was not within the original scope and design of this experiment to obtain this type of information. However, the evidence presented here clearly indicates that a significant proportion of these mosaics are due to X-chromosome elimination.

The data on the frequency of mosaics listed in table 1 show and support the work of other investigators, that the addition of Minute-n gene increases the

frequency of mosaics. In addition, these data show that the frequency of the Minute-n mosaics may be further increased by decreasing the temperature. The opposite effect is found among the non-Minute mosaics. The frequency tends to increase by increasing the temperature. The explanation underlying these facts does not at present seem clear. There is no doubt, however, that this effect is closely connected with the problem of the thermal effects on the physical structure of certain regions of the X-chromosome, especially since the M-n locus is located very close to the spindle attachment.

SUMMARY

Many advantages have been pointed out concerning the use of mosaics as a technique in studying the different phases of developmental processes, such as, genic action in relation to embryonic induction and cell differentiation. More specifically, an attempt has been made in this report to give some information on the action of the Bar series in reducing the size of the eye in relation to the general problem of embryonic induction and cell differentiation.

From the data presented here the following conclusions have been derived: (1) The different members of the Bar series reduce the eye size by quantitatively controlling the mechanism of cell division of the anterior portion of the eye anlage. (2) A change in temperature produces a change in the frequency of mosaics; in the Minute-n mosaics the frequency is higher at 20°C than at 25°C; in the non-Minute-n the frequency is higher at 25°C than at 20°C.

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AN INTERPRETATION OF THE OBSERVED AMOUNT OF RECOMBINATION IN INTERCHANGE HETEROZYGOTES OF BARLEY[1]

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IN an individual heterozygous for an interchange between two non-homologous chromosomes, the observed amount of gene recombination in an interstitial region depends on the frequency of the respective centromere disjunction types and upon the chiasma (crossover) frequency expected for the two interstitial regions of the interchange complex. An interstitial region is considered to be the chromosome segment between the centromere and the point of interchange. LAMM (1948) presented the formulas from TOMETORP's unpublished analysis to express this dependency of the recombination measurement upon these factors; however, only the chiasma frequency in the interstitial region being studied was considered. The amount of gametic sterility expressed by an interchange heterozygote as measured by the frequency of spore abortion also is a function of the frequency of centromere disjunction types and the chiasma frequencies in the two interstitial regions. BURNHAM (1948) recognized this dependency and noted that a species could have primarily alternate segregation yet exhibit sterility of 50 percent. HANSON and KRAMER (1949) have presented and discussed the relationships for the observed amount of recombination and the amount of sterility in terms of the centromere orientation frequencies and the chiasma frequencies in both interstitial regions. Based on the formulas presented, the observed recombination and the amount of gametic sterility were interpreted, and the interrelationship between these two factors was discussed.

In the analysis presented by HANSON and KRAMER (1949), the assumption was made that at the first division only the theoretically possible 2–2 segregations from the chromosomes of the interchange complex occur, with the segregation types being independent of crossing over. In this paper these segregation types will be referred to as alternate, adjacent 1, and adjacent 2 (MCCLINTOCK 1945) which correspond to the centromere disjunction types A, B and C, respectively, used in the 1949 analysis. Alternate and adjacent 1 segregations involve homologous centromere disjunction at division I and are differentiated on the condition that alternate or adjacent chromosomes pass to the same pole. Adjacent 2 segregation involves nondisjunction of homologous centromeres and disjunction of the translocated pieces. Also, the assumption was made that only single crossing over occurs within an interstitial region, but that the occurrence of crossovers in the two interstitial regions is

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independent. Based on these conditions, the conclusions were drawn that as long as the frequency of alternate and adjacent 1 segregations are equal, the percent recombination measured for an interchange heterozygote would be that expected for the normal diploid, while the amount of gametic sterility is a function of the adjacent 1 and adjacent 2 segregation frequencies. When the frequency of alternate segregation exceeds that of the adjacent 1 segregation, a reduction of observed recombination would be anticipated as compared to that normally expected. Under unequal frequencies of alternate and adjacent 1 segregations the observed recombination, as well as the amount of gametic sterility, depends upon the frequency of the centromere disjunction types and upon the chiasma frequencies in the two interstitial regions. The reduction in recombination results from the tendency of recombination chromatids arising from crossovers in an interstitial region to be associated with the abortive spores. If double crossing over occurs, crossover chromatids also may be recovered with alternate segregation (BURNHAM 1950). The effects of double crossing over would appear to be limited in interchange heterozygotes exhibiting partial sterility since the longer interstitial regions also are associated with sterility near fifty percent (table 7, HANSON and KRAMER 1949).

The actual frequency of the segregation types in barley interchanges would be difficult to determine. Cytological determinations of the frequencies of open and zigzag rings at metaphase could be made with the assumption that zigzag rings lead to alternate segregation. This assumption may not be true since zigzag rings may be oriented to give adjacent segregation (KOLLER 1944). BURNHAM (1950), through use of the nuclear organizer region which is carried on chromosome 6 in corn, was able to determine the segregation frequencies in some of the translocations involving the sixth chromosome. The relative frequency of the alternate segregation may be inferred from the sterility relations presented by HANSON and KRAMER (1949). The sterility for the interchanges studied in barley averaged about 25 percent, indicating that alternate segregation may predominate in barley. If this is true, the observed recombination in the interchange heterozygotes should be greatly reduced. The purpose of this paper is to present a group of data demonstrating the reduction of recombination for measurements made in the interchange heterozygotes of barley.

MATERIAL AND METHODS

HANSON and KRAMER (1949) have presented the genetic analysis from F_2 data of two interchanges in barley referred to as accessions 300 (Minnesota C–1420) and 301 (Minnesota C–1432) and (1950) have completed the analysis of the 301 interchange with F_3 data. Interchanges 300 and 301 were shown to involve linkage groups I and IV and IV and VI, respectively. These interchanges together with the genetic stocks used in the studies were obtained from DR. C. R. BURNHAM, Department of Genetics and Plant Breeding, University Farm, St. Paul, Minnesota. Five additional interchange stocks also were obtained from DR. BURNHAM and studied at the Purdue University

Agricultural Experiment Station. Of the additional interchange stocks, accession 302 (Minnesota C–1456) was found to involve linkage group IV. Since three genetic markers are available for linkage group IV (*K,k* and *Lg₃,lg₃* in the 293 stock and *Bl,bl* in the 296 stock), the complete analysis of interchanges 300, 301, and 302 and the measurements of the recombination between the *K* and *Lg₃* loci should be made in view of possible reduction in recombination in interchange heterozygotes.

ROBERTSON *et al.* (1947) have summarized the linkage studies in barley. Based on this summary, the genetic stocks and their genotypes used in this study are:

Purdue accession	Genetic markers used	Respective linkage groups
293	VV, BB, nn, KKSegLg₃lg₃*	I, II, III, IV
294	KK, ss, SegXcxc	IV, V, VI
295	KK, ss	IV, V
296	BlBl, RRss, SegYcyc	IV, V, VII
297	wxwx**	VII

* HANSON, W. D., and H. H. KRAMER (1949) place the locus of *Lg₃,lg₃* in the IV linkage group.

** Unpublished data of O. J. WEBSTER (personal correspondence), G. A. WIEBE and R. G. SHANDS place *Wx,wx* in the VII linkage group.

The F_2 data from the crosses involving interchange stock 302 with the genetic stocks were obtained in the field during the summer of 1948. Classification for partial sterility was made on the basis of seed set. For additional information in the F_3 for interchanges 300 and 302, dominant F_2 plants were selected randomly from the partial sterile and normal classes, and the F_3 progeny rows were grown from each selected plant during the summer of 1949. The analysis for linkage will be made by the method of maximum likelihood as adapted for segregating data involving an interchange by HANSON and KRAMER (1950).

Data were presented (1949) identifying the recessive seedling lethal factor pair *Lg₃,lg₃* with the fourth linkage group in barley. Since the locus of *Lg₃,lg₃* appeared to be near the interstitial regions of interchanges 300 and 301, F_3 data were obtained for linkage between *Lg₃,lg₃* and the 300 and 301 interchange breaks and between *Lg₃,lg₃* and *K,k* in material segregating for these interchanges. Data in the F_3 also were obtained for linkage between *Lg₃,lg₃* and the 302 interchange break.

PRESENTATION OF DATA

The analysis of linkage data involving partial sterility

The summary of the genetic data for interchange 302 crossed to the five genetic testers is presented in table 1. The data include the F_2 and F_3 data from these crosses together with the designations a to h corresponding to

TABLE 1

Linkage data in the F_2 and F_3 for interchange 302 involving partial sterility and the genetic markers.

Cross	Aa	SSA– a	NA– b	SSaa c	Naa d	Total	SSAA e	SSAa f	NAA g	NAa* h
293 × 302	Non-segregating lg_3									
	Vv	88	79	26	27	220				
	Bb	97	70	17	36	220	17	80	41	28
	Nn	83	74	31	32	220				
	Kk	98	76	16	30	220	10	72	39	16
293 × 302	Segregating lg_3									
	Vv	18	7	3	4	32				
	Bb	20	5	1	6	32				
	Nn	14	5	7	6	32				
	Kk	18	3	3	8	32				
	Lg_3lg_3	22	10	0	0	32	1	21	8	2
294 × 302	Non-segregating xc									
	Kk	190	129	34	71	424				
	Ss	168	142	56	58	424				
295 × 302										
	Kk	104	83	27	59	273				
	Ss	97	113	34	29	273				
296 × 302	Non-segregating yc									
	Blbl	150	95	19	52	316				
	Rr	132	123	37	24	316				
297 × 302										
	Wxwx	130	117	40	28	315				
	Nn	132	113	38	32	315				

* SS and N will be used in this paper to designate the classification of partial sterile and normal, respectively.

the observed numbers in the formulas of tables 3 and 4 of HANSON and KRAMER (1950).

The first steps in the analysis for linkage in such data are to test the hypothesis of independent assortment between the characters being studied and to determine the homogeneity of various sources of data. This can be done very quickly through the application of the scoring relationships summarized in formulas B and C page 563, HANSON and KRAMER (1950). The mechanics for calculating χ^2 values for linkage and for population homogeneity have been outlined and will not be presented in this paper.

The χ^2 values for linkage in the individual sources of data, for linkage in the total sample, and for population homogeneity between partial sterility and the genetic markers are given for interchange 302 in table 2. The χ^2 for linkage in the total sample is based on the total score and information for the combined sources of data and is the test for independence between the interchange break and the factor pair as measured in the combined data. The linkage groups of the 302 interchange complex are clearly differentiable. Linkage is shown between the interchange break and *B,b* of linkage group II and *K,k*, *Bl,bl* and Lg_3,lg_3 of linkage group IV. Since the interchange break is linked with Lg_3,lg_3, a recessive seedling lethal, data in which this factor is

TABLE 2

The χ^2 values for linkage in the individual sources of data, for linkage in the total sample, and for population homogeneity between partial sterility and the genetic markers for interchange 302.

Cross	Source	Aa	Source χ^2	d. f.	Total χ^2	d. f.	Homogeneity χ^2	Homogeneity d. f.
293 N. Seg.	F_2	Vv	.22	1				
293 Seg. lg_3	F_2	Vv	2.04	1	.01	1	2.25	1
293 N. Seg.	F_2	Bb	10.69**	1				
293 N. Seg.	F_3(SS)	Bb	10.91**	1				
293 N. Seg.	F_3(N)	Bb	21.14**	1				
293 N. Seg. lg_3†	F_2	Bb	9.36**	1	40.58**	1	2.16	2
293 N. Seg.	F_2	Nn	.22	1				
293 Seg. lg_3	F_2	Nn	.37	1				
297	F_2	Nn	.00	1	.21	1	.38	2
293 N. Seg.	F_2	Kk	6.20*	1				
293 N. Seg.	F_3(SS)	Kk	16.50**	1				
293 N. Seg.	F_3(N)	Kk	34.94**	1				
293 Seg. lg_3†	F_2	Kk	9.36**	1				
294 N. Seg.	F_2	Kk	23.25**	1				
295	F_2	Kk	16.71**	1	87.75**	1	9.85*	4
293 Seg. lg_3	F_2	Lg_3lg_3	4.50*	1				
293 Seg. lg_3	F_3(SS)	Lg_3lg_3	8.21**	1				
293 Seg. lg_3	F_3(N)	Lg_3lg_3	9.80**	1	21.12**	1	1.39	2
294 N. Seg.	F_2	Ss	.81	1				
295	F_2	Ss	1.17	1	.00	1	1.98	1
296	F_2	Blbl	25.01**	1	25.01**	1		
296	F_2	Rr	.95	1	.95	1		
297	F_2	Wxwx	.56	1	.56	1		

† Omitted because interchange point was linked to a lethal seedling factor.
* Significant at 5 percent level.
** Significant at 1 percent level.

segregating in the 293 × 302 cross cannot be used with the formulas presented for the determination of linkage between the interchange point and the factor pairs *K,k* and *B,b*, respectively, and have been omitted from the population homogeneity determinations. The χ^2 value for population homogeneity in the *K,k* group is just significant at the five percent level. This set of data will be considered homogeneous and the linkage intensity between *K,k* and the interchange break will be computed on the combined data. The sources of data for each of the other three factor pairs demonstrating linkage are homogeneous and combinable.

Additional data in the F_3 together with the F_2 data presented by HANSON and KRAMER (1949) are summarized in table 3 for linkage between *V,v* and interchange 300 and between Lg_3,lg_3 and the interchanges 300 and 301.

The analysis for linkage in these data is made in table 4. The evidence for linkage between the 300 interchange break and *V,v* is supported by F_3 data

TABLE 3

Linkage data in the F_2 and F_3 between V,v and Lg_3lg_3 and the respective interchange breaks.

Cross	Aa	SSA– a	NA– b	SSaa c	Naa d	SSAA e	SSAa f	NAA g	NAa h
293 × 300	Non-segregating lg_3								
	Vv	57	51	11	17	11	47	27	21
293 × 300	Segregating lg_3								
	Lg_3lg_3	122	60			7	115	58	2
293 × 301	Segregating lg_3								
	Lg_3lg_3	96	47			1	95	44	3

with the χ^2 for linkage in the total sample being highly significant. Heterogeneity within the three sets of linkage data exists only in the 293 × 301 cross. The heterogeneity of data in this cross could be explained by misclassification for partial sterility in a few of the F_2 plants. Since the linkage is almost complete in these data, the scores are large, and only a small error in classification could create heterogeneity. Since all three sources of data are estimating linkage intensities close to zero, the data will be combined for an estimate of the recombination value.

The next step in the analysis of genetic data is to obtain the best estimate of linkage intensity for each set of combined data. The method of scoring using the maximum likelihood formulas has been used in this study to determine the recombination values. HANSON and KRAMER (1950) have outlined the details of this analysis which will not be given in this paper. The summary of linkage intensities between the interchange complexes and the different

TABLE 4

The χ^2 values for linkage in the individual sources of data, for linkage in the total sample, and for population homogeneity involving V,v and Lg_3lg_3 and partial sterility for the respective interchanges.

Cross	Source	Aa	Sample χ^2	d. f.	Total χ^2	d. f.	Homogeneity χ^2	Homogeneity d. f.
293 × 300	Non-segregating lg_3							
	F_2	Vv	1.41	1				
	F_3(SS)	Vv	5.39*	1				
	F_3(N)	Vv	11.34**	1	15.61**	1	2.53	2
293 × 300	Segregating lg_3							
	F_2	Lg_3lg_3	21.11**	1				
	F_3(SS)	Lg_3lg_3	41.80**	1				
	F_3(N)	Lg_3lg_3	24.31**	1	84.48**	1	2.74	2
293 × 301	Segregating lg_3							
	F_2	Lg_3lg_3	16.79**	1				
	F_3(SS)	Lg_3lg_3	45.06**	1				
	F_3(N)	Lg_3lg_3	76.94**	1	127.50**	1	11.29*	2

* Significant at the 5 percent level.
** Significant at the 1 percent level.

TABLE 5

Summary of the linkage intensities between the interchange points 300, 301, and 302 and the respective gene pairs together with the observed numbers for each group of data.

Interchange	Aa	Linkage group	Observed numbers								p ± S.E.
			a	b	c	d	e	f	g	h	
300	Vv	I	57	51	11	17	11	47	27	21	.20 ± .033
300	Kk	IV	402	239	27	216					.06 ± .011*
300	BlBl	IV	168	125	23	57					.17 ± .032*
300	Lg_3lg_3	IV	122	60			7	115	58	2	.026 ± .0080
301	Kk	IV	207	140	23	87	13	138	57	36	.12 ± .014**
301	BlBl	IV	173	111	28	64					.16 ± .059*
301	Xcxc	VI	105	89			4	101	65	24	.08 ± .014**
301	Lg_3lg_3	IV	96	47			1	95	44	3	.014 ± .0072
302	Bb	II	97	70	17	36	17	80	41	28	.17 ± .023
302	Kk	IV	392	288	77	160	10	72	39	16	.17 ± .016
302	BlBl	IV	150	95	19	52					.14 ± .030
302	Lg_3lg_3	IV	22	10			1	21	8	2	.05 ± .028

* From HANSON AND KRAMER (1949).
** From HANSON AND KRAMER (1950).

factor pairs demonstrating linkage is presented in table 5. For convenience, the observed numbers in each class also are given. The linkage intensities previously presented are included in this summary.

Linkage studies with the factor pair Lg_3,lg_3

The original parent 293 was segregating for the character green *vs.* light green seedlings (Lg_3,lg_3) which was identified with linkage group IV in barley. Additional data in the F_3 are presented in table 6 for linkage between the loci of this recessive seedling lethal and *K*,*k*. Linkage data between Lg_3,lg_3 and *K*,*k* are available for the interchange heterozygotes 300 and 301 and for a set of data (standard) not involving an interchange in linkage group IV. The observed numbers are referred to as e to n, respectively.

KRAMER and BURNHAM (1947) have presented the maximum likelihood formulas for the determination of the recombination value for such data involving two factor pairs segregating from a cross made in repulsion. Since the doubly heterozygous F_2 plants were not separated into the classes k(AB/ab) and i(Ab/aB), the two classes have been combined into one class desig-

TABLE 6

Linkage data in the F_3 for Lg_3lg_3 with K,k.

Cross	$KKLg_3Lg_3$ e	$KKLg_3lg_3$ f	$KkLg_3lg_3$ g	$KkLg_3lg_3$ n	$kkLg_3Lg_3$ l	$kkLg_3lg_3$ m
293 × 300	0	8	12	98	53	7
293 × 301	1	5	8	65	36	13
Standard	2	6	8	74	31	7

nated as n. Through the application of the formulas for F_3 data from doubly dominant F_2 plants (AB) and from singly dominant F_2 plants (aB), the linkages between *K,k* and Lg_3,lg_3 are calculated to be as follows:

Source	p ± S.E.
300	.080 ± .015
301	.119 ± .023
Standard	.105 ± .020

DISCUSSION

The reduction of recombination in an interchange heterozygote as compared to that normally expected involves the loss of a certain proportion of the crossover chromatids arising from chromatid exchange in an interstitial region. The dependency of the recovery of the crossover chromatids upon the frequency of centromere disjunction types and upon the chiasma frequencies in the interstitial regions has been illustrated in figure 1 and in table 7 of HANSON and KRAMER (1949). Directly associated with the observed recombination is the amount of gametic sterility exhibited by the interchange heterozygote. In order to observe a gametic sterility of less than fifty percent, the frequency of the alternate segregation is expected to exceed the sum of the other segregation frequencies, and the chiasma frequencies in the two interstitial regions must be less than unity. Under these conditions the crossover chromatids tend to be associated with the abortive spores, and the failure to recover the crossovers reduces the observed recombination. This reduction occurs only for linkage measurements made within an interstitial region. A recombination value estimated for a chromosome segment will be affected only by the portion contained within an interstitial region.

The interchange heterozygotes used in this study averaged about 24 percent abortive pollen. A critical analysis of the linkage measurements involving the fourth linkage group should present evidence of reduced recombination in the interstitial regions of the barley interchange heterozygote.

From the linkage intensities presented, the genetic maps shown in figure 1 can be made for the fourth linkage group, where T represents the position of the break in the translocated chromosome.

ROBERTSON *et al.* (1947) list 44.0, 24.7, 40.6 and 22.0 for the percent recombination between *K,k* and *Bl,bl*. From the linkages of *K,k* and *Bl,bl* to T_{302}, the larger pair of recombination values appear to be the more acceptable measurement of linkage between the two loci. It is evident from these linkage relationships that all three of the breaks must lie between the *K* and the *bl* loci and that an interstitial region would exist between either *K,k* or *Bl,bl* and the respective translocation points. If the *K* and *bl* loci are 40–44 map units apart, there must have been a considerable degree of loss of crossover chromatids arising from chromatid exchange in the interstitial regions of the 300, 301 and 302 complexes, unless appreciable asynapsis had occurred. Further, the centromere must lie to the right (as diagrammed) of T_{300}, T_{301} and T_{302} such that the linkage measurements of *Bl,bl* to the respective interchange

breaks involve an interstitial region. In the sequence, T_{300}, T_{301} and T_{302}, the breaks are progressively to the right (as diagrammed) of the *K* locus, these three measurements differing significantly (the standard errors of the difference are .018, .019 and .021 for the comparisons of .06–.12, .06–.17, and .12–.17, respectively). Yet, there is little corresponding decrease in the linkage measurements between the *bl* locus and the interchange breaks which would be expected if an interstitial region were involved in these linkage measurements. The linkage measurements between *K* and Lg_3 in the 301 and the standard stocks are comparable, and the combined estimate of recombination is .111 ± .015. In the 300 source the Lg_3 locus is apparently in the interstitial region between T_{300} and the *bl* locus which would account for the re-

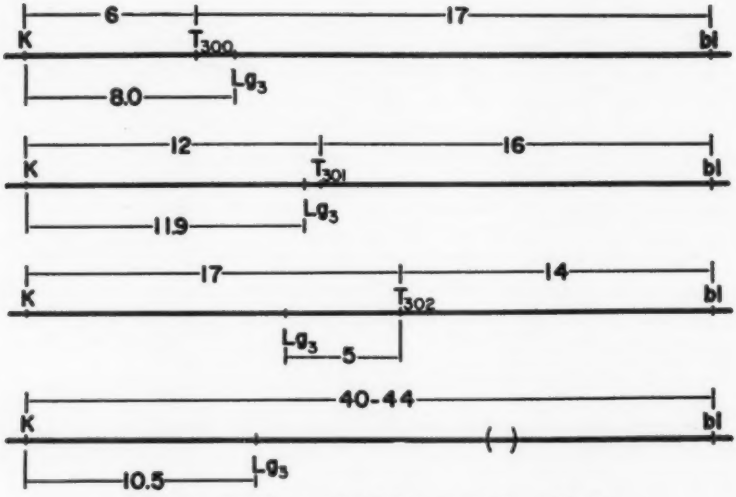

FIGURE 1.—Genetic maps for linkage group four of barley.

duction in the observed recombination. The probability for the test of significance between .080 and .111 is about .14.

The relative position of the centromere could be postulated. The sequence would be *K*–Lg_3–T_{302}–centromere–*bl*; however, the sequence also may be *K*–Lg_3–T_{302}–*bl*–centromere. The 302 translocation point is used for reference. If the latter sequence were to be accepted, interchange 300 would be expected to have a sterility appreciably higher than the observed 25 percent.

HANSON and KRAMER (1949) have noted that the frequency of the alternate segregation must be very high in barley to account for the high degree of masking observed. If this is true, interchanges ranging from almost complete fertility to 50 percent sterility may be expected in barley. A detailed study of the different interchanges was made by the examination of pollen

obtained from heads selected within a two-day period from crosses of the interchange stalks to the genetic tester 296. The sterility ranged from 21.0 ± 1.8 to 31.6 ± 1.5 for the group of interchanges. The .95 confidence intervals for the sterility of the three interchanges presented in this study were as follows:

Interchange	Percent sterility
300	25.4 ± 1.9
301	25.4 ± 2.9
302	21.0 ± 1.8

In interchange 302 low sterility would be expected (depending upon the chiasma frequency in the second interstitial region) since the break is postulated to lie nearer the centromere in linkage group IV than the other two interchange breaks, thus giving a low chiasma frequency for this region. The data on recombination and sterility agree with the expectations for primarily alternate segregation in barley.

SUMMARY

The analysis of interchange 302 was presented based on F_2 and F_3 data together with the analysis of additional F_3 data for interchanges 300 and 301. The linkage groups involved in interchange complexes 300, 301, and 302 are I and IV, IV and VI, and II and IV, respectively. Linkage intensities between the interchange breaks and the respective genetic markers were given.

The locus of the seedling lethal factor pair Lg_3,lg_3 was established in linkage group IV between the *K* and *bl* loci. Linkages of the factor pair with *K*,*k* and with the interchange breaks 300, 301 and 302 were given. The Lg_3 locus lies about 11.1 map units from the *K* locus, being in the non-translocated segment carrying the *bl* locus in interchange 300 and in the translocated segment carrying the *K* locus in interchanges 301 and 302.

Data were presented and discussed for linkage measurements in the fourth linkage group in barley. A satisfactory interpretation of these linkage intensities involved the assumption that a high proportion of the crossover chromatids arising from crossovers within an interstitial region were associated with the abortive spores. Thus, the observed recombination for the interchange heterozygote would be appreciably smaller than that expected. The complete analysis entailed an interpretation of the interrelationship between the condition of reduced recombination and the amount of sterility exhibited by the interchange heterozygote. The information was used to postulate gene position in relation to the interchange breaks and the centromere.

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